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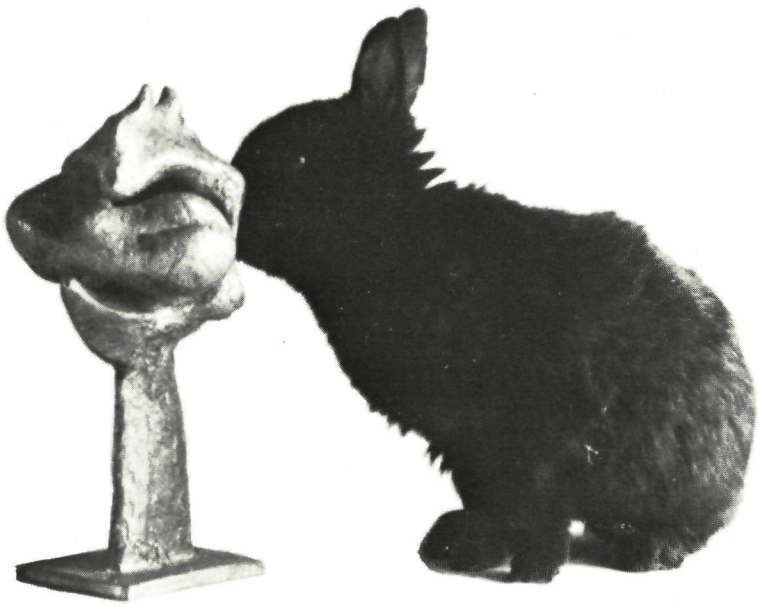
TREATMENT OF REJECTION OF HUMAN KIDNEY GRAFTS WITH RABBIT ANTITHYMOCYTE GLOBULIN



Andries J. Hoitsma

Sculptuur in brons "De Bedreigde Nier" door Joep Nicolas-van Ronckenstein samen met een ATG-producent.

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Promotor: Prof. dr. R.A.P. Koene

Aan Sheneke

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CONTENTS

CHAPTER 1

INTRODUCTION

Renal transplantation is now a generally accepted procedure for the treatment of advanced chronic renal failure. Patient survival after transplantation has steadily increased especially as a consequence of the more judicious use of corticosteroids in the prevention and treatment of graft rejection. After an initial improvement in the first years of transplantation, graft survival rates have remained stable in most centers during the last decade. When only patients who received a kidney from a cadaveric donor after 1978 are included, the one year patient and graft survival rates in the Sint Radboud Hospital were 92 and 71 percent respectively.

Most failures develop in the first three months after transplantation and 80 percent of the graft failures are caused by rejection[1]. For immunosuppression, prednisone and azathioprine are the drugs most commonly used in these patients, but apparently this regimen cannot always prevent an acute rejection. More importantly, high doses of steroids are also not always sufficient to reverse an established rejection. To improve graft survival, more effective immunosuppressive agents are obviously required. This led us to evaluate the efficacy of antilymphocyte serum (ALS) in human renal transplantation.

The notion that it might be possible to prepare a serum specifically active against one type of cell was first put forward by the great Russian zoologist Metchnikoff (1899)[2], who used the material to investigate the cellular basis of inflammation. During the subsequent 50 years animal studies layed the basis for most of our current knowledge of ALS, but it was not until Waksman and his colleagues[3] demonstrated in rats that ALS suppressed delayed hypersensitivity responses, that its clinical use was actually considered. The application of the agent to organ transplantation, seemed a logical approach, particularly since there was growing evidence for an important role of lymphocytes in allograft rejection. In the years thereafter ALS proved to be remarkably effective in prolonging allograft survival in most animal models. An extensive survey of the effects of ALS in experimental animals was given by Lance et al[4].

Despite a host of experimental studies the mechanism of the immunosuppressive activity of ALS is still unclear. ALS seems to exert its action by inducing a depletion of peripheral blood T lymphocytes, but it is not known how this depletion actually occurs. Despite the fact that ALS can cause complement-mediated lysis of lymphocytes in vitro, there is no convincing evidence that the same mechanism is operative in vivo[5]. Results of animal studies suggest that circulating lymphocytes coated with ALS are efficiently opsonized by macrophages within the liver and in lymphoid organs[6,7].

The clinical effectiveness of ALS has been even more difficult to evaluate. The reasons for this are many. Of course, there is a natural reluctance to inject large volumes of foreign protein into man because of the danger of allergic reactions. More importantly, however, it has been difficult to standardize the preparation of ALS. As a consequence, ALS preparations can differ widely in potency, and the situation is further complicated by the fact that this potency is very hard to evaluate, because in vitro tests that correlate with its in vivo effectiveness are not available. Prolongation of skin graft survival in monkeys is considered to be the only reliable parameter. Undoubtedly, these difficulties have led to the use of inadequate dosage schedules in several studies. There has been no general agreement on the species in which to raise the antiserum or on the type of antigen that would produce the most potent and least toxic antibody. In most centers that have tested ALS the number of patients treated (mostly a mixture of cadaveric and related transplantations) were insufficient to enable evaluation of the agent in a prospective randomized trial, and this led to multicenter trials with their inherent problems. Over the years several factors that are now known to influence graft outcome became apparent (blood transfusions, acute tubular necrosis, HLA typing, etc.). In the early studies with ALS no attempts were made to balance for these factors between test and control groups, which is a further complicating factor in the evaluation of the activity of ALS. In studies performed in the past, ATG has been almost exclusively used as an adjuvant immunosuppressant, administered together with azathioprine and prednisone from the day of transplantation. Although a reduced incidence of early rejection episodes has usually been observed with such protocols, sig-

nificant differences in long term survival rates between patients randomly assigned to treated and control groups were only found in a few studies.

The use of the abovementioned protocol was based on the assumption that ALS was especially effective in preventing the onset of an immune response, but that it interfered less well with an ongoing response in experimental animals. It was, however, not completely ineffective in this regard, which made it worthwhile to investigate its effectiveness in the clinical situation. Over the last five years ALS has, therefore, been used by some groups for the treatment of acute renal graft rejection and in most studies the short term results were encouraging. Obvious advantages of this approach as opposed to prophylactic treatment are that the agent is administered at the time it is most needed and that a considerable number of patients who never experience a rejection will not unnecessarily be exposed to the risks inherent to treatment with a foreign immunosuppressive protein.

The initial results obtained with ALS in the treatment of acute rejections of renal grafts were encouraging enough to justify a systematic study. We have, therefore, started a prospective randomized trial in April 1979. This enabled us not only to obtain information on the effectiveness of ALS in the immediate reversal of acute rejections, but also to decide whether this new approach would have a beneficial influence on the long term survival of patients and grafts.

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CHAPTER 2

ALLOCATION OF PATIENTS TO TREATMENT GROUPS WHEN NOT ALL BALANCING FACTORS ARE KNOWN TO THE COORDINATING CENTER

Henk JJ van Lier, Philip van Elteren, Andries J Hoitsma, Robert AP Koene

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ABSTRACT

The allocation of patients to treatment groups while balancing for risk factors is usually done by a coordinating center when all risk factors are known. In a clinical trial comparing two treatments of acute rejection of renal allografts some risk factors become available to the physician only at the moment of the diagnosis of the rejection. This diagnosis is often made out of office hours whereas immediate start of treatment is essential. It is shown here that the coordinating center is still able to control the allocation of patients to treatment groups if a small number of risk factors is only known to the physician.

In 1979 a single center clinical trial was started to study the effects of the treatment of first acute rejection of renal allografts occurring within three months after transplantation. Treatment with antithymocyte globulin (ATG) was compared to conventional treatment with prednisone. The first results have been published elsewhere[1,2].

The trial had to conform to the following criteria:

1. Patient and physician had to be kept unaware of the possible treatment prior to allocation. Ignorance after the allocation was not possible because ATG had to be administered by infusion and prednisone was given orally. This was also not necessary because the outcome of the treatment could not be influenced (graft failure).
2. Patients had to be allocated to treatment groups while balancing for factors which might reflect the severity of the rejection or would influence the outcome of the treatment. The method of Taves[3] was initially used for this purpose.

This method consists of several steps and is equivalent to the range method of Pocock and Simon[4]. At first, each risk factor is divided in categories. Then, in a table, here called the Taves scheme, the number of patients in both treatment groups and in each category of the risk factors are recorded. Freedman and White[5] developed a simplified method that is followed here for the description of the next steps. At the entry of a new patient the Taves scheme is inspected with regard to the categories of this patient. Then the number of categories with an overrepresentation of the first treatment (n_1) and with an underrepresentation (n_2) are noted. Categories with an equal number of patients are left out. If n_1 is smaller than n_2 the new patient will be assigned to the first treatment group. If n_1 is larger than n_2 the patient will be assigned to the second treatment group. In case n_1 equals n_2 the new patient will be assigned with probability 0.50 to one of the treatment groups.

In Table 1 the factors and their respective categories are listed that were considered to be of importance to establish the severity of the

Table 1 Balancing factors in a clinical trial comparing ATG and prednisone in treatment of acute renal allograft rejection

Factor		Categories
1	Age of patient	≤ 40 / > 40 years
2	Sex of patient	male / female
3	Sex of donor	male / female
4	Blood group patient	0 / non-0
5	Blood group donor	0 / non-0
6	Blood transfusions	none / 1 / 2 or more / unknown
7	Previous transplants	none /1 / 2 or more
8	Renal osteodystrophy	yes / no
9	HLA-AB mismatches	none / 1 on A-locus / 1 on B-locus / 2 or more
10	HLA-antibodies	none / ≤ 50% / > 50%
11	HLA-DR mismatches	0 / 1 / 2 / unknown
12	B-cell antibodies	negative / positive / unknown
13	Interval transplantation to rejection	≤ 12 / > 12 days
14	Body temperature at day of rejection	≤ 37.2 / > 37.2°C
15	Dialysis within four days prior to rejection	yes / no

rejection. Of the factors mentioned the first 12 are known from the date of the transplantation. Only the factors "interval transplantation to rejection", "body temperature at the day of rejection" and "dialysis within four days prior to rejection" become available at the time of diagnosis (rejection), but only to the physician. The coordinating center that controls the allocation is only staffed during office hours. It was expected that the diagnosis would often be made late in the afternoon. During weekends the coordinating center would also not be able to allocate patients to treatment groups. In the treatment of acute rejection it is essential that the treatment is started as soon as possible to minimize damage to the kidney. Therefore, it was expected that even during office hours the coordinating center would not be able to allocate patients on time.

THE SOLUTION

For this organizational problem the following solution was found:

1. In the Taves scheme of the clinical trial patients with their 15 risk factors are only admitted after the development of an acute rejection.
2. For each of the eight combinations of the categories of the risk factors 13 to 15 of a transplanted patient whose risk factors 1 to 12 are known (and who has not yet experienced an acute rejection) an allocation is performed without changing the Taves scheme. The resulting treatments are stated in sealed forms and the coordinating center places these forms in a file on the transplantation department. This is done for all patients immediately after the transplantation.
3. At the time of the diagnosis of the rejection the appropriate form of the patient is opened. The number of the patient, the actual risk factors 13 to 15 and the treatment group are passed on to the coordinating center as soon as possible.
4. The coordinating center updates the Taves scheme, repeats the procedure at point 2 for all patients who have not yet shown signs of acute rejection and replaces the sealed forms.

5. If sealed forms are not available (e.g. in case of an acute rejection occurring soon after transplantation) the physician will open the envelope with the lowest number of a set of sealed and numbered envelopes. The envelopes contain cards indicating one of the two possible treatments chosen at random.
6. When forms are made for a patient more than 12 days after the transplantation the risk factor 13 (interval transplantation to rejection) is known. Thus, in these cases only four instead of eight forms are required.
7. For each patient forms have to be made or changed during three months after transplantation. After this period patients are no longer admitted to the trial.

EXAMPLE

The above procedure is illustrated in a simplified situation with only four risk factors (6, 13, 14, 15). The first risk factor becomes available on the day of the transplantation, the other three only at the moment of an acute rejection. We start from the situation that 17 patients have been allocated to the ATG group and 16 to the Prednisone group (for numbers of patients in the categories see Table 2). Forms must be made for a patient who has had two blood transfusions prior to the transplantation and whose transplantation has taken place more than 12 days ago.

In each category to which the patient belongs the difference is determined between the number of patients of that category who have been treated with ATG and those treated with prednisone. Subsequently, each of these differences is scored +1 when positive, 0 when zero, and -1 when negative and the scores are added. If the sum of the scores is positive the new patient will be allocated to the Prednisone group and, in the case of a negative sum, to the ATG group. Should the sum be zero, the patient will be allocated at random to a treatment group with probability 0.50. If at the day of rejection the new patient has a body temperature of 37.2°C or less and has been dialyzed, then the sum of

Table 2 Example with risk factors 6, 13, 14, and 15
Numbers of patients already allocated to each treatment group and scores of the differences

Factor		Category	Number of patients A*	Number of patients P*	Diff.*	Score
6	Blood transfusions	0	1	2	-1	-1
		1	13	11	2	+1
		2 or more	3	3	0	0
		unknown	0	0	0	0
13	Interval transplantation to rejection	⩽ 12 days	9	7	2	+1
		> 12 days	8	9	-1	-1
14	Body temperature at day of rejection	⩽ 37.2°C	11	9	2	+1
		> 37.2°C	6	7	-1	-1
15	Dialysis within four days prior to rejection	yes	2	1	1	+1
		no	15	15	0	0
Total			17	16		

* A = ATG group, P = Prednisone group, Diff = difference in number of patients in ATG and Prednisone group

the scores will be positive and the patient will receive prednisone. This treatment is then stated in the sealed form for this particular situation. Table 3 shows the four combinations of categories and the contents of the forms for the patient considered.

DISCUSSION

The method described here was mainly developed because in the clinical trial considered three risk factors could not be made available to the coordinating center on time. The risk factor "interval transplantation to rejection" has often been reported to be of prognostic value[6,7]. There is a clear indication that rejections occurring soon after transplantation are more serious and often become irreversible. The risk factor "dialysis within four days prior to rejection" was taken into account because diagnosis of an acute rejection and patient management are much more difficult after dialysis. The risk factor "body temperature at day of rejection" is less well established, but the authors have a strong indication that patients with a high body temperature have a more severe rejection.

These three factors with the risk factors already known at the day of the transplantation had to be distributed equally in both treatment groups. At first, the method of Taves[3] was used for this purpose. Non-balancing allocation methods like simple randomization were discarded because differences in numbers of patients would easily become too large, especially for analyses to be performed in subgroups (in subgroups of 20 patients the probability is more than 0.01 that a treatment group contains four or fewer patients; in subgroups of 40 patients this figure is 12).

After the publication of the paper by Begg and Iglewicz[8] the same simulations were performed also using the standardized variance method, an obvious extension of the variance method of Pocock and Simon[4]. With the variance method the scores are multiplied by the absolute difference in

Table 3 Scores and contents of forms in example for a patient with two blood transfusions and an interval after transplantation of more than 12 days

Factor		Category	Combination 1	2	3	4
6	Blood transfusions	2 or more	0	0	0	0
13	Interval transplantation to rejection	> 12 days	-1	-1	-1	-1
14	Body temperature at day of rejection	⩽ 37.2°C	1	1		
		> 37.2°C			-1	-1
15	Dialysis within four days prior to rejection	yes	1		1	
		no		0		0
Sum of scores			1	0	-1	-2
Content of form			P*	C*	A*	A*

* A = allocation to ATG group, P = allocation to Prednisone group, C = allocation to treatment groups at random with probability 0.50. The resulting allocation is stated inside the form.

the categories, and greater weight is given to larger differences. With standardization the scores are also divided by the number of patients in the categories, and greater weight is given to small subgroups than to large subgroups. The simulations revealed the smallest imbalances in the categories and in the total number of patients for the standardized variance method. Therefore the actual allocation method was changed accordingly. The scores were also multiplied by a positive number that was larger for risk factors that were expected to have greater influence on the outcome of the treatment. Neither change did affect the applicability of the allocation procedure described in this paper, but would unnecessarily complicate the presentation.

Meier[9] states as an objection to the use of balancing allocation methods that it is sometimes difficult or impossible to classify a patient prior to treatment and mentions the problems arising from a wrong classification (should the patient be reassigned to the right category or not?). By using the method proposed in this paper the risk of a wrong classification is reduced. This method can be used when the time between the diagnosis or the measurement of risk factors and the commencement of the treatment is short. It is most easily applied when at the moment of diagnosis few risk factors need to be introduced whereas the remaining risk factors have already been measured at an earlier stage. Such a situation not only occurs during renal allograft rejection but also in heart operations (e.g. the risk factor "extent of occlusion in a coronary artery" can not be determined accurately by angiogram prior to the operation but becomes available during the operation) and operations for the removal of tumors (e.g. size and stage of the tumors). If there are many unknown risk factors, however, this method will soon become impractical.

A problem that one may encounter is the situation in which many patients are in the interval between transplantation and rejection. Allocation methods like the method of Taves and the (standardized) variance method have the property that the numbers of patients in both treatment groups are nearly always equal. When an imbalance in the total number of patients exists the allocation of the next patient will almost surely counteract this imbalance. In that case the same treatment will be

stated in the sealed forms of many patients. If the physician has to open forms for second and following patients before he can contact the coordinating center, he will often be able to predict to which treatment group these patients will be allocated. This would seriously increase the possibility of selection bias. This problem is not likely to occur in our clinical trial with one or two transplantations a week and a probability of acute rejection in the first three months of 0.60 to 0.70. However, should this situation occur regularly, then the use of the sealed and numbered envelopes (i.e. simple randomization) for second and following patients is recommended. Because these patients are subsequently admitted to the Taves scheme any possible lack of balance can generally be compensated for afterwards.

In our clinical trial four to six patients were concomitantly at risk for an acute rejection. For preparing, sealing and placing the forms an average time of 30 to 60 minutes a week was required, because a computer was used to update the Taves scheme and to print the forms. The use of a personal computer by the physician would circumvent a procedure as described here, but would also increase the possibility of selection bias by the physician and require the training of the physicians and other members of the staff of the transplantation department.

It is obvious that in a multicenter clinical trial the coordinating center has to pass information to all local coordinating units after each rejection. These local units should be equiped with adequate printing facilities. The procedure described so far is rather time consuming. In clinical trials with a large number of patients or a large number of unknown risk factors the following adaptation might be considered. Two sets of sealed and numbered envelopes are available at every transplantation center, the first one containing cards indicating one of the two treatments chosen at random, the second one containing cards with "yes" or "no". The number of yes cards has to be larger than the number of no cards. After each rejection in the clinical trial a sealed form is (re-)placed at every transplantation center containing the number of patients in the categories (the Taves scheme) or the scores. When a rejection occurs at a center, the physician opens this sealed form and performs the calculations required for the allocation proce-

dure. In the case of no imbalance an envelope is taken from the first set. When a treatment is underrepresented an envelope is taken from the second set. If the card indicates yes the patient will be allocated to this prescribed group. Otherwise, he will be allocated to the other group. The risk factors and the treatment group are passed on to the coordinating center as soon as possible. This adaptation reduces the number of forms to be made by the local coordinating units or the coordinating center. However, it requires some arithmetic by the busy physician and enlarges the knowledge of the physician about the state of the clinical trial. To prevent selection bias the number of yes cards has to be chosen very carefully, but the imbalances in the categories and in the total number of patients are likely to increase with fewer yes cards.

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CHAPTER 3

TREATMENT OF ACUTE REJECTION OF CADAVERIC RENAL ALLOGRAFTS WITH RABBIT ANTITHYMOCYTE GLOBULIN

Andries J Hoitsma, Paul Reekers, Johan G Kreeftenberg, Henk JJ van Lier, Peter JA Capel, Robert AP Koene

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TREATMENT OF ACUTE REJECTION OF CADAVERIC RENAL ALLOGRAFTS WITH RABBIT ANTITHYMOCYTE GLOBULIN

ANDRIES J HOITSMA PAUL RFEKERS JOHAN G KREEFTENBERG HENK J J VAN LIER
PETER J A CAPEL AND ROBERT A P KOENE

Department of Medicine Division of Nephrology Laboratory of Transplantation Serology and Statistical Support Center Sint Radboudziekenhuis University of Nijmegen and Rijksinstituut voor de Volksgezondheid Bilthoven The Netherlands

In a prospective randomized single-blind trial, we compared the effectiveness of rabbit antithymocyte globulin (RATG) in the treatment of acute renal graft rejection with the results of treatment by high oral doses of prednisone Twenty recipients of cadaveric kidneys were included in each group In the RATG group, the prednisone dose was not increased and a dose-by rosette protocol was used to keep T cell levels between 50 and 150/mm³ In this group 15 of the 20 patients responded to the treatment One of these patients lost her kidney afterward because of a technical failure In five patients rejection was irreversible despite a subsequent course of high-dose prednisone orally In the prednisone group, 13 patients showed a good response, but 3 of them only after a subsequent course of RATG The remaining seven patients underwent nephrectomy before a course of RATG could be given One patient in this group died of septicemia In either group there were six second rejection episodes, but they developed 2 2 months later in the RATG group All second rejection episodes were treated with the alternative regimen and all patients responded to this treatment Renal function after 6 months was similar in both groups Less infections occurred in the RATG group Prior to rejection, there were no differences in concentrations of peripheral T cells between both groups Treatment of acute rejections with RATG is an effective and safe procedure which is steroid sparing

The question whether treatment with antithymocyte globulin improves kidney graft survival in man is still a matter of debate Recent results of Thomas et al (*1*) have shown that the efficacy of antithymocyte globulin (ATG) depends on its potency as measured by prolongation of skin graft survival in primates Furthermore there is evidence that the source of ATG is also of importance equine ATG being less immunosuppressive than RATG (*2*) In most trials the administration of ATG is started immediately after transplantation whereas high doses of steroids are administered when rejection occurs There are only a few reports on the efficacy of ATG treatment started at the time of acute rejection and again the available data are conflicting Two studies (*3 4*) showed no benefit of this form of treatment whereas in two others the only advantages were the occurrence of fewer second rejection episodes (*5*) and a more rapid reversal of rejection (*6*) A recent study showed for the first time a better graft survival in patients treated with ATG for their rejection (*7*) However these results are somewhat difficult to assess since in all studies mentioned ATG was administered in addition to standard treatment of rejection with high doses of steroids Shield et al (*8*) were the first to show the effectiveness of ATG when added at the time of rejection without a concomitant increase of the steroid dosage In this study only recipients of related donor kidneys were included Hardy et al (*9*) added ATG when rejections were not responsive to a 3 to 5 day course of standard antirejection treatment and found a better graft survival in the ATG treated group as compared with a control group All of the ATG preparations that were used in the above mentioned trials were of equine origin and therefore probably of moderate or low potency

We have tried to obviate some of the difficulties encountered in previous investigations by using a high potency RATG as the single agent in the treatment of first rejections of cadaveric grafts and by comparing the results in a randomized trial with the effects of high oral doses of prednisone

MATERIALS AND METHODS

Forty recipients of a cadaveric renal allograft who experienced a rejection episode were included in our trial Related donors and diabetic patients were excluded Only patients with first rejection episodes within 3 months after transplantation were admitted to the protocol Twenty eight recipients who did not experience a rejection within the first 3 months served as an additional control group Acute rejection was diagnosed according to the standard criteria (increase of serum creatinine sodium retention oliguria weight gain hypertension proteinuria tenderness of the graft) Biopsies were only done when second rejection episodes occurred Twenty patients were treated with RATG and 20 received high doses of steroids orally RATG that was prepared in the Rijksinstituut voor de Volksgezondheid according to the method described by Kreeftenberg (*2*) induced a skin graft survival of 20 to 30 days in cynomolgus monkeys whereas the survival time was 7 to 8 days in untreated animals A commercial horse ATG preparation (ATGAM the Upjohn Company) induced a graft survival of 10 to 11 days in this model In rhesus monkeys skin graft survival with RATG was 50 days For the allocation of the patients to the different treatment groups the minimization method of Taves (*10*) was used balancing the following variables (with different weights) sex age blood group of the recipient number of blood transfusions number of mismatches for HLA A B and DR antigens presence of lymphocytotoxic antibodies and B cell antibodies number of previous transplants time of onset of the acute rejection after transplantation and body temperature at the day of diagnosis Until the diagnosis of acute rejection was made all patients were treated similarly prednisone was started on the day of transplantation in a dose of 100 mg daily and tapered in periods of 5 days to 75 50 40 30 and 25 mg The azathioprine dose was 1 5 mg/kg body wt when the creatinine clearance was less than 25 ml/min and 3 mg/kg at higher clearances The dose was also decreased when the leu

kocyte count was less than 4000/mm^3 or when the thrombocytes were less than 100,000/mm^3 In the RATG group the acute rejection was treated with a 3-week course of RATG i v The initial dose was 4 mg/kg and additional doses were between 2 and 7 mg/kg with a total frequency that varied from three to nine doses, depending on the peripheral T cell level that we tried to keep between 50 to 150/mm^3 (*11*) The prednisone dose was not raised in the RATG group, except for the addition of 50 mg of prednisolone to the first and 25 mg to each following RATG infusion to avoid acute side effects In the prednisone group the oral dose of prednisone was raised to 200 mg/day when acute rejection was diagnosed This dose was tapered in 3- or 5-day periods to 100, 75, 50, 40, 30, and 25 mg Intravenous steroid pulses were not used Treatment was switched to the alternative regimen if there was no improvement of renal function after 10 days or if a deterioration of renal function occurred after completion of the treatment course The day of reversal of a rejection episode was defined as the second of 3 consecutive days that the serum creatinine decreased

Peripheral T cells were determined with the 2-amino ethylisothiouronium bromide technique (*12*) Briefly, in the Hemalog D, lymphocytes were counted in whole blood, and recounted after separation with Ficoll The percentage of rosette-forming cells was then determined, and absolute T cell levels were calculated from these data The RATG level was determined with a radial immunodiffusion technique and antibodies to RATG with an agglutination assay Complement components C3, C4, and factor B were measured by radial immunodiffusion For the detection of circulating immune complexes, a modification of the C1q binding assay described by Zubler et al (*13*) was used For statistical evaluation the following methods were used Mann-Whitney U test, test of Kruskal-Wallis, Wilcoxon matched pairs test and χ^2 test Differences were considered significant if P levels were less than 0 05

RESULTS

Table 1 shows that the RATG and prednisone groups were similar for factors that might influence graft survival The mean serum creatinine level, measured on the day when the acute rejection was diagnosed, was 3 8 mg/100 ml in the prednisone group and 3 6 mg/100 ml in the RATG group, also indicating that there were no great differences between both groups in the severity of rejection The results obtained with the two treatment protocols are given in Table 2 and Figure 1

In the RATG group only two patients showed no response at all, whereas there were five such patients in the prednisone group Besides the five immunological failures in the RATG group, there was one technical failure This was in a patient who developed a leakage from the renal pelvis attributable to obstruction after she had responded to the RATG treatment The mean reversal of the rejection occurred 1 1 day earlier in the RATG group but this was not significant There were six second rejections in either group that were, according to the protocol, treated with the alternative regimen The mean interval between first and second rejections was 2 2 months longer in the RATG group Figure 1 shows that a second rejection episode within the first 3 months after transplantation occurred only once in this group, whereas this was the case four times in the prednisone group For the comparison of renal function in both groups, we included only patients who had experienced a single rejection Serum creatinine levels were not different between the two groups The 28 patients who never developed

TABLE 1 Patient data

	RATG	Prednisone
Sex male/female	10/10	13/7
Age <40/>40 years	9/11	13/7
Blood group O/non-O	8/12	7/13
Blood transfusion		
1	6	7
2 or more	14	12
Unknown	0	1
HLA AB mismatches		
0	5	5
1	6	6
2 or more	9	9
HLA DR mismatches		
0	9	9
1	8	5
2	1	2
Unknown	2	4
HLA antibodies		
none	11	13
<50%	6	4
>50%	3	3
B cell antibodies		
Negative	16	15
Positive	3	2
Unknown	1	3
Previous transplants none/1 or more	17/3	17/3
Body temperature at day of rejection ≤37 2/>37 2 C	7/13	7/13
Interval from transplantation to rejection ≤12/>12 days	10/10	9/11

TABLE 2 Response to antirejection treatment and subsequent course

	RATG	Prednisone
No reversal (no of patients)	2	5
Immunological failures (no of patients)	5	7
Mean graft survival of failures (months)	2 1	1 5
Mean day of reversal	3 8	4 9
Second rejection episode	6	6
Interval between first and second rejection (months)	5 3	3 1
Mean serum creatinine level at 6 months (mg/100 ml)	1 1	1 0

a rejection had a mean serum creatinine level of 1 2 mg/100 ml after 6 months, and this was not different from the two treatment groups

Peripheral T cell levels were measured in all patients In the RATG group we tried to adjust the doses of RATG such that the T cell levels were between 50 and 150/mm^3 At the onset of rejection, mean T cell levels of both treatment groups were similar (RATG, 870, prednisone, 821/mm^3), and not significantly different from the level of the control group of 28 patients without a rejection (795/mm^3) In the RATG group the mean T cell level fell significantly during treatment, whereas this was not the case in the prednisone treated group (Fig 2)

Since we adjusted the RATG dose to the peripheral T cell level, it is not surprising that there were great differences in the doses between individual patients This is shown in Figure 3, which also illustrates that reversal of the rejection occurred promptly in most cases There was no relation between cumulative RATG dose and the reversal day Figure 3 also shows that the rapidity of the reversal provides no information on the ultimate outcome of the transplantation Two patients with a

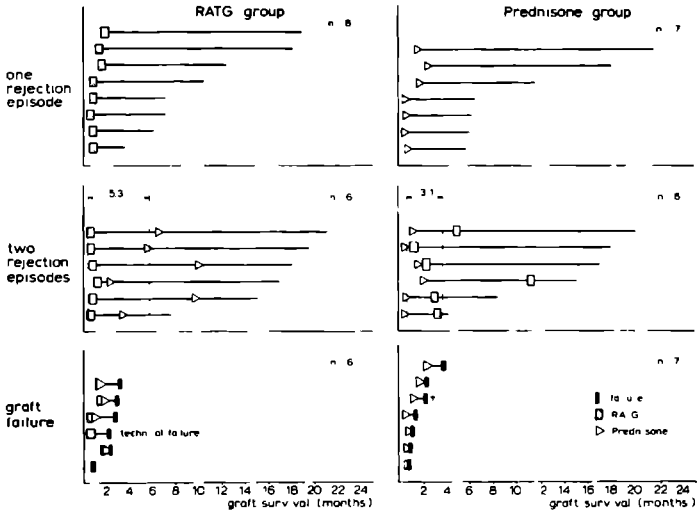

FIGURE 1 Graft survival in both treatment groups The patients with two rejection episodes were treated with the alternative regimen The vertical broken lines indicate the mean onset of first and second rejections The numbers between these lines refer to the mean intervals (months) between first and second rejections In the prednisone group one patient died as a result of septicemia after abdominal surgery (†)

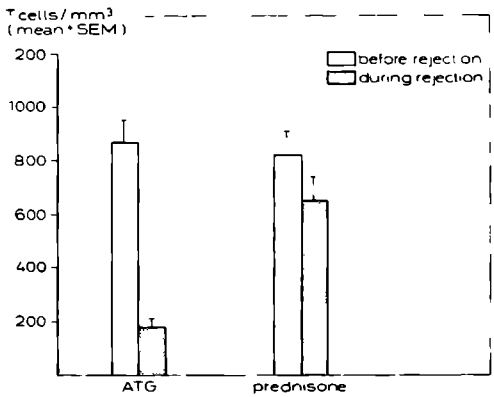

FIGURE 2 Mean peripheral T cell levels before and during treatment in both groups

rapid reversal developed renal failure, whereas in three with very slow reversals graft outcome was good

Differences in elimination of RATG might reflect its in vivo activity and therefore we measured RATG levels in the serum There were no differences between RATG levels in the serum of patients who responded without further rejections (mean 121 μg/ml), in the patients who had a second rejection (mean 95 μg/ml) and in the treatment failures (mean 118 μg/ml) In two patients who responded to a single antirejection course RATG levels became zero despite continued administration This was also the case in one patient of the failure group and in three patients who later developed a second rejection In only one patient we were able to detect antibodies against rabbit immunoglobulins These antibodies developed 1 week after completion of the RATG course This patient ultimately lost her kidney Complement levels were slightly below normal in all patients that received transplants, but there were no significant changes during the rejection treatment Levels of immune complexes remained either negative or unchanged in all patients

The only adverse reactions to the RATG treatment were chills and fever which occurred almost exclusively during the first infusion Two patients developed slight and transient arthralgias during the treatment course In none of the patients did RATG treatment have to be discontinued because of side effects

In the prednisone group there were 29 urinary tract infections in 15 patients We found significantly less urinary tract infections in the RATG group (16 in 9 patients) but the incidence was high again in the group with no rejection (34 in 19 patients) The frequencies of all other infections, including cytomegalovirus infections were not significantly different There were four cytomegalovirus infections in the prednisone group and five in the RATG group One patient in the prednisone group died as a result of septicemia

The mean cumulative steroid dose per patient after 3 months was 4275 mg in the prednisone group and 3255 mg in the RATG group Only patients who sustained adequate renal function for at least 3 months were included in this comparison irrespective of whether they had one or two rejection episodes

DISCUSSION

Treatment of acute rejection with RATG has two obvious advantages as compared with a protocol of prophylactic treatment started from the day of transplantation First, patients without a clinically apparent rejection do not receive unnecessary treatment with the foreign protein That this is a considerable number of patients is illustrated by our finding that 28 of a total of 68 patients had no rejection during the period of study Second if a patient is treated from the day of transplantation he runs the risk of having already formed antibodies

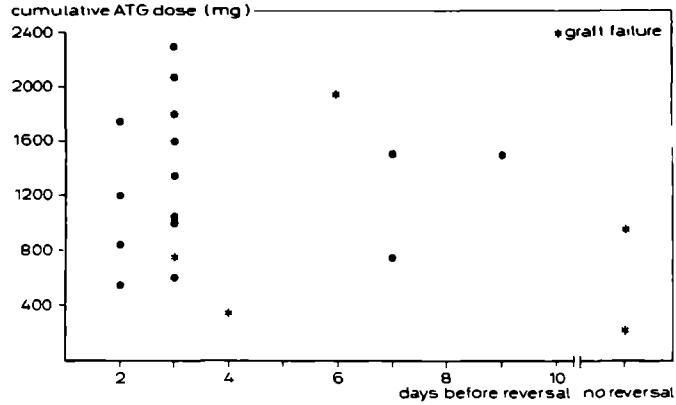

FIGURE 3 Relation between cumulative RATG dose and promptness of rejection reversal Two patients showed no reversal (right panel)

against the rabbit proteins at the time that rejection becomes evident, thus making further treatment less effective

Another problem with ATGs from different sources is their variable potency Generally, horse ATG seems to be less efficient than RATG That was the reason we chose the latter ATG source Even with ATG of this species, it remains important to establish the immunosuppressive activity by performing skin graft studies in primates The preparation that we have developed compares favorably with ATGs used by other groups (*1*) since it produced a skin graft survival time of 50 days in a rhesus monkey and 20 to 30 days in the less sensitive cynomolgus model

Although the results are preliminary and conclusions about long-term survival cannot be drawn because of the relatively short observation time and the limited number of patients treated as yet, several data that are important for clinical decision making have already emerged It is clear that reversal of acute rejection is possible in most cases by treatment with RATG and without raising the dose of steroids This is in accordance with the results in two other recent studies, in which horse ATG was used as the immunosuppressant drug (*8, 9*) Shield et al (*8*) only studied rejections in living related donors in whom rejections may be generally easier to control because of better matching as compared with cadaver donors The results of Hardy et al (*9*) are somewhat difficult to interpret, since they started treatment in patients who did not respond to 3 or 5 days of treatment with high doses of prednisone and graft irradiation Although their data are impressive, it cannot be completely excluded that in some of their patients the favorable result was attributable to a delayed response to the prednisone treatment and not to the administration of ATG In our approach these problems could be obviated by including only patients with cadaveric grafts and by studying the efficacy of RATG as the sole agent in a prospective randomized trial In the RATG group there were more patients who showed reversal of their rejection, whereas the time of rejection reversal tended to be shorter All but one of the patients with immunological graft failure in the RATG group received a subsequent full course of prednisone treatment without effect In most of these patients a full treatment course with RATG could not be given because of leukopenia and thrombocytopenia. In all of the failures in the prednisone group, the kidney grafts had to be removed before a subsequent RATG course could be given The excellent response to RATG in all six patients of the prednisone group who developed a second rejection shows that second rejection episodes are also responsive to RATG Furthermore, our results indicate that the interval between first and second rejection episodes was longer in the RATG group As anticipated, the RATG protocol was steroid sparing

We did not find a correlation between the onset of acute rejection and the level of peripheral T cells RATG treatment caused a large drop in T cell levels that occurred in most cases immediately after the start of the treatment This drop was not seen in the prednisone group (Fig 2) In neither group could the T cell levels be used to predict the final outcome of the treatment procedure

Bieber et al (*14*) carried out a study on the clearance rates of RATG after a full course of RATG had been given to cardiac transplant recipients They found that survival was better in patients with long RATG half lives Since we did not give a continuous course of RATG with a constant dose, we could not study the serum half-life, but still some conclusions may be drawn from our measurements of RATG levels We found no differences in mean RATG levels between failures and successful treatments We even found that in two responsive patients levels became zero despite continued administration of RATG The same was found in three patients who later on suffered from second rejections These findings throw doubt on the assumption that measurement of RATG levels might be helpful to establish the ideal dose or to predict the outcome of the treatment The use of a dose-by-rosette protocol led to great differences in RATG doses between individual patients The mean dose of RATG and the frequency of its administration was lower in the patients with immunological graft failure, but this was caused by the fact that three patients in this group reacted to the first RATG doses with severe thrombocytopenia or leukopenia, which made it necessary to interrupt the treatment We are as yet unable to decide whether the hematological changes were merely signs of irreversible rejection or whether the rejection occurred because the thrombocytopenia and leukopenia made it impossible to give an adequate treatment with RATG It is conceivable that patients who needed only small

amounts of RATG to keep their T cell levels between 50 and 150/mm might fall in the group of fast responders However we found no correlation between the RATG dose and day of reversal (Fig 3)

The chills and fever which usually occurred in the first hour of the first RATG infusion are most likely caused by the sudden massive cytolysis of lymphocytes and are not a hypersensitivity reaction to the foreign protein as such since they usually did not recur during subsequent infusions

The steroid sparing effect of the RATG treatment will have advantages with regard to the many complications in which steroids play a role Long term observations will certainly give information about the incidence of osteonecrosis in both groups In this short term study we found only indications for the occurrence of less urinary tract infections in the RATG group However we hesitate to draw conclusions from this finding since the incidence in the group with no rejection was at least as high as in the prednisone group It has been suggested that cytomegalovirus infections occur more frequently after ATG but in our study such an increased incidence was not found (*15*)

Our results show that treatment with RATG in a dose by rosette protocol is an effective and safe method to treat rejection The results are at least as good as those with high oral doses of prednisone whereas the RATG protocol is definitely steroid sparing

Acknowledgments We are indebted to Drs J Berden J Rosier R Tuggeler F Huysmans and H van Roermund for advice and assistance in the treatment of the patients and to Dr H Wessels Dr P Faaber and J Paardekoper for expert technical assistance

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CHAPTER 4

TREATMENT OF SECOND AND LATE RENAL ALLOGRAFT REJECTION WITH RABBIT ANTI-HUMAN THYMOCYTE GLOBULIN.

Andries J Hoitsma, Paul Reekers, Johan G Kreeftenberg, Henk JJ van Lier, Peter JA Capel, Robert AP Koene

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Treatment of Second and Late Renal Allograft Rejection With Rabbit Anti-Human Thymocyte Globulin

A. J. Hoitsma, P. Reekers, J. G. Kreeftenberg, H. J. J. van Lier, P. J. A. Capel, and R. A P. Koene

IN HUMAN renal transplantation, antithymocyte globulin (ATG) has been used for many years. Initially, ATG was administered from the day of transplantation in most clinical trials, and the results of these trials are conflicting. An increasing number of recent studies deal with the efficacy of ATG when started at the moment of a first rejection.[1-13] Although treatment protocols differ widely, most studies have shown some beneficial effect of this form of treatment. The information on the efficacy of ATG treatment, when used for second rejections, late rejections (> 3 months after transplantation), or steroid-resistant rejections is still scanty.[3,5,12,13] Moreover, in all these studies ATG was used as an adjunctive agent in combination with high doses of steroids so that the effect of ATG by itself is difficult to assess We have therefore treated second, late, or steroid-resistant rejections with ATG as the sole antirejection agent.

MATERIALS AND METHODS

All but one patient received a cadaver kidney Treatment consisted of prednisone and azathioprine from day of transplantation When a first rejection occurred within 3 months after transplantation, the oral prednisone dose was raised to 200 mg/day and tapered in periods of 3–5 days to 25 mg/day in about 3 weeks Anti-rejection treatment with RATG consisted of an initial dose of 4 mg/kg body weight, further doses ranging from 2–7 mg/kg depending on the peripheral T-cell levels We tried to keep these levels between 50 and 150/μl [14] The prednisone dose was not raised during this latter treatment

RATG was prepared according to the method described by Kreeftenberg [15] Peripheral T cells were determined with the amino ethyl-isothiouronium bromide (AET) rosetting technique[16] and absolute figures were estimated with the Hemalog-D RATG-levels were determined with a radial immunodiffusion technique and antibodies against RATG with an agglutination assay A rejection was diagnosed according to standard criteria and was proven by a renal biopsy in most cases The day of rejection reversal was defined as the second of three consecutive days that the serum creatinine decreased

Twelve patients received RATG infusions for a second rejection episode that occurred within 3 months after transplantation (early second rejections) Six of these patients had experienced a steroid-resistant first rejection, which was defined as a failure of renal function to improve after 10 days of treatment with high doses of prednisone, or as a repeated fall of renal function immediately after completion of a treatment course There were 15 patients who received RATG treatment for a rejection occurring more than 3 months after grafting (late rejections) Twenty patients treated for their first acute rejection with RATG served as a control group

RESULTS

Early Second Rejections

The 12 patients who were treated with RATG infusions for their second rejection episode within the first 3 months after transplantation had all received the conventional high doses of prednisone during their first rejection episode. The results of the subsequent RATG treatment are given in Table 1, and they show that compared with the patients who received RATG for their first rejection episode (controls), the response was less impressive: reversal occurred more slowly and there were more patients in whom renal function did not improve. The mean dose of RATG was similar in both groups, as was the total number of gifts On the other hand mean RATG levels and T-cell levels were higher during treatment. Administration of RATG in the second rejection group often had to be

From the Department of Medicine, Division of Nephrology, the Laboratory of Transplantation Serology, and the Statistical Support Centre, Sint Radboudziekenhuis, University of Nijmegen, and Rijksinstituut voor de Volksgezondheid, Bilthoven, The Netherlands

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Reprint requests should be addresed to A J Hoitsma, Department of Medicine, Division of Nephrology, Sint Radboudziekenhuis, University of Nijmegen, The Netherlands

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Table 1 Response to Anti Rejection Treatment and Subsequent Course

	Second Rejection (<3 months)	Late Rejection (>3 months)	Control Group*
Number of patients	12	15	20
Mean serum creatinine at day of rejection (mg/100 ml)	2 9	2 2	3 6
Mean day of reversal	4 5	—†	3 8
No reversal of serum creatinine (number of patients)	4	11	2
Mean dose of RATG (mg)	1375	2005	1203
Mean number of RATG gifts	6 0	8 3	5 8
Mean RATG level (μg/liter)	171	196	121
Mean T cells during treatment (per μl)	312	300	190
Mean serum creatinine (mg/100 ml) 6 months after treatment	1 6	1 6‡	1 1

*Patients who received RATG for their first rejection

†Not calculated because of low number of patients

‡Mean level of 12 patients Three patients had a graft failure

stopped temporarily because of thrombocytopenia and leukopenia The mean serum creatinine level at 6 months after anti-rejection treatment was lower in the control group One patient lost his kidney 9 months after transplantation All other kidneys are still functioning (follow-up ranging from 5 months to 2 years) Adverse reactions to RATG were less frequent in the second rejection group Only half of them showed transient fever during the first infusion This occurred in 90% of the control patients Other reactions were not seen Antibodies against RATG were not found in the second rejection group In 10 patients a renal biopsy was performed before the start of the treatment of the second rejection There were three purely vascular rejections, six combined vascular and interstitial rejections, and one interstitial rejection The patient who lost his kidney had a combined rejection In the subgroup of six patients in whom the first rejection had been steroid-resistant, we reversed the rejection with RATG in all cases

Late Rejections

There were 15 patients in this group, 8 of them had received the conventional high dose of prednisone for their first acute rejection within 3 months after transplantation The remaining seven patients had not experienced a rejection during the first 3 months The mean interval between transplantation and rejection was 15 months (range 3–94 months) The results obtained in this group are also given in Table 1 The mean day of reversal was difficult to assess, because only 4 of the 15 patients showed a reversal of their serum creatinine Eight patients needed subsequent treatment with high doses of prednisone after completion of the RATG course, whereas there were only two such patients in the group of early second rejections The mean RATG dose and the mean number of gifts was higher, but despite these higher doses we could not sufficiently depress the T-cell levels (mean level 300/μl) The mean RATG level was higher compared with the control group Three patients lost their kidneys by immunologic failure, which occurred at a mean of 3 5 months after treatment In the remaining 12 patients, the mean serum creatinine at 6 months after treatment was higher than in the control group All patients underwent a renal biopsy One patient had a purely vascular rejection, 10 patients had combined rejection, and 4 patients had interstitial rejection All patients who eventually lost their kidney had a combined rejection

Antibodies against RATG were not found The side effects of RATG in the late rejection group were also less Half of the patients

Table 2 Incidence of Infections Within Three Months After Treatment

	Second Rejections (<3 months)	Late Rejections (>3 months)	Control Group
Number of patients	12	15	20
Urinary tract infections	6	1	29
Other infections	2	2	8
Cytomegalovirus infections	2	2	5
Prior cytomegalovirus infections	3	3	—

suffered from fever during the first infusion Also in this group, five patients showed slight signs of serum sickness In the control group, we found 18 patients with fever and 2 with serum sickness In no case did these side effects necessitate discontinuation of the RATG treatment

The infection incidence in all groups is compared in Table 2 In both the early and late rejection group there were fewer urinary tract infections and other infections compared with the control group If prior infections with cytomegalovirus are taken into account, the incidence was the same for all groups

DISCUSSION

Our results show that RATG treatment of second rejections that occur within 3 months after transplantation is successful in most cases, even if they have been resistant to high oral prednisone treatment An exception must be made for those steroid-resistant rejections that are so severe that the kidney is lost before subsequent RATG treatment can be instituted [10 11] In the few earlier reports about treatment of second rejections, ATG was always used as an adjunctive agent [3 5 12 13] Our finding that the beneficial effect can be obtained without raising the prednisone dose is especially encouraging It is worthwhile to mention that RATG treatment can still be effective when it is given some time after the onset of the rejection We have treated two patients in whom rejection was unfortunately diagnosed 10 days after its onset Despite this delay, there was a prompt response to RATG treatment and complete reversal was attained Both patients now have normal renal function at 6 and 25 months after transplantation, respectively It is also remarkable that in biopsy-proven rejections of predominantly vascular type treatment was so successful It raises doubt about the generally held opinion that vascular rejection is almost always resistant to treatment

In the late-rejection group, the results were rather disappointing In most cases we could not induce reversal of the serum creatinine It was striking that in only three patients did the rejection go on to complete renal failure In the remaining patients, renal function stabilized Probably, RATG treatment halted the rejection in some of these patients, but it is difficult to assess whether this holds for all patients since eight of them received a subsequent course with high oral prednisone

In both treatment groups it was more difficult to depress the T-cell levels with RATG than in the control group In the early second-rejection group, this was caused mainly by the high incidence of leukopenia and thrombocytopenia, which prevented us from giving RATG more frequently In the late-rejection group, the patients seemed to be more resistant to RATG treatment, since T-cell levels remained relatively high, despite the fact that the mean total dose of RATG administered was much higher than in the control group The reasons for this apparent resistance are not clear

The incidence of infections in both treatment groups was lower than in the control group These figures are somewhat difficult to interpret, because it can be expected that the infection rate will decline late after transplantation and both treatment groups received RATG at a later stage than the control group Still, the absolute figures are surprisingly low

Together with the almost complete absence of adverse reactions, this indicates that RATG treatment is a relatively harmless procedure.

We conclude that RATG is not only effective in reversing first rejections as we have shown earlier,[10,11] but that early second rejections are equally sensitive. For the present this latter conclusion must be limited to patients who received steroids for their first rejection. We have not enough systematic experience in patients who received RATG for both their first and second rejections, although our preliminary results in a few cases suggest that RATG is often still effective when given for the second time in the same patient. Late rejections, on the other hand, showed almost no response. In this group of patients, primary treatment with prednisone might be a better choice.

ACKNOWLEDGMENTS

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CHAPTER 5

HLA-DRw6 AND TREATMENT OF ACUTE REJECTION WITH ANTITHYMOCYTE GLOBULIN

Andries J Hoitsma, Paul Reekers, Henk JJ van Lier, Jan CM van Rens,
Robert AP Koene

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SUMMARY

The influence of DRw6-antigen on graft survival was studied in a single center study in 223 recipients of a cadaveric kidney. Although graft survival in 148 DRw6-negative recipients was not significantly different from that in 75 DRw6-positive recipients, the percentage of patients without a rejection episode in the first three months after grafting was significantly less in the DRw6-negative recipients (P=0.03). In DRw6-positive patients who had received RATG as the first antirejection treatment, graft survival was significantly better than in prednisone-treated DRw6-positive recipients. In the DRw6-negative patients RATG treatment gave also better results, but these differences were not significant. When RATG treated patients were excluded from the analysis the difference in graft survival between DRw6-negative and positive patients became apparent (P=0.03). These findings show that the negative influence of the DRw6-antigen present in the recipients is counterbalanced by the beneficial effect of RATG treatment for first rejection episodes.

INTRODUCTION

Matching for DR-antigens has been reported to improve graft survival in renal transplantation[1,2]. Recently, Hendriks et al showed that the presence or absence of the DRw6-antigen in the recipient is an even more important factor[3,4]. DRw6-negative recipients had a significantly better one-year graft survival than DRw6-positive patients. They also found that HLA-DR matching improved renal allograft survival only in the DRw6-positive recipients. In DRw6-negative patients a slightly beneficial effect of HLA-DR matching was observed, but this was not significant. On initial analysis of graft survival, patient survival, and effectiveness of HLA-DR matching in our DRw6-positive and DRw6-negative patients, we could not confirm these findings. We have therefore, studied whether differences in treatment protocols for rejection might be responsible for our failure to find a negative influence of the DRw6-antigen on graft survival in DRw6-positive recipients.

PATIENTS AND METHODS

In this single center study only patients grafted after January 1, 1978 were studied, because HLA-DR typing was not performed routinely before that time. All cadaveric transplantations of which HLA-DR types of both donor and recipient were known, were admitted. All patients had received at least one blood transfusion before transplantation and had been followed for at least three months after grafting.

From the day of transplantation all patients received prednisone in a dose of 100 mg daily with tapering in periods of 5 days to 75, 50, 40, 30, and 25 mg. The azathioprine dose was 1.5 mg/kg body weight when the creatinine clearance was less than 25 ml/min and 3 mg/kg at higher clearances. The dose was also decreased when the leucocyte count was less than $4,000/mm^3$ or when the thrombocytes were less than $100,000/mm^3$. Acute rejections were treated in two different ways, according to an earlier described randomization protocol[5]. In one group of patients the oral dose of prednisone was raised to 200 mg/day and

tapered in 3- or 5-day periods to 100, 75, 50, 40, 30, and 25 mg. Intravenous steroid pulses were not used. In the other group acute rejections were treated with a three-week course of rabbit antithymocyte globulin (RATG)[5,6]. The initial dose was 4 mg/kg and additional doses were between 2 and 7 mg/kg depending on the peripheral T cell level that we tried to keep between 50 to 150/mm^3. The prednisone dose was not raised in these patients except for the addition of 50 mg of prednisolone to the first and 25 mg to each following RATG infusion to avoid acute side effects. If the patient experienced a second rejection episode or if the first rejection responded insufficiently to treatment, the alternative treatment was given. Graft failure was defined as a return of the patient to hemodialysis, regardless of whether the cause of failure was immunological or non-immunological. Patients who died were also counted as graft failures, even if the graft was functioning at the time of death. Patient failures include all patients who died even if the cause of death was unrelated to the transplantation.

Typing for HLA-DR antigens (DR1-DR10, MB1-MB3, MT2-MT3) was done with the NIH standard lymphocytotoxicity assay on enriched B-lymphocyte suspensions, obtained by rosetting with aminoethyl-isothiouronium bromide (AET) coated sheep red blood cells. The antigen DRw6 was serologically defined by positive reactions with MB1 and MT2 sera. Recipients were all typed in the same laboratory and their HLA types were all confirmed by typing of family members. The gene frequencies of the recipient's DR-antigens in the whole group and the two treatment groups are given in Table I. As a result of the family typing the number of unknown DR-antigens was kept to a minimum. The HLA type of the kidney donors was defined by comparing the results of two independent typing laboratories. The frequency of unknown DR-antigens was 0.11 for the whole group. If at a given locus one antigen was unknown, the donor was considered to be homozygous for this specificity. The HLA-AB match grade was calculated as follows: 1 point with 1 mismatch at the A-locus, 2 points with 1 mismatch at the B-locus, and 3 points with 2 or more mismatches. One point was assigned for each DR mismatch.

The actuarial survival curves were estimated with the survival-program of SPSS. The log rank test according to Mantel[7] was applied to test

Table I. Gene frequencies of DR antigens in the recipients

	Total Group (n=223)	RATG Group (n=66)	Prednisone Group (n=77)
DR 1	0.11	0.14	0.06
DR 2	0.15	0.09	0.16
DR 3	0.13	0.13	0.12
DR 4	0.14	0.16	0.10
DR 5	0.14	0.13	0.16
DR 6	0.17	0.20	0.21
DR 7	0.10	0.09	0.15
DR 8	0.03	0.03	0.02
DR 9	0.00	-	0.01
DR 10	0.00	-	-
Blanks	0.03	0.04	0.01

differences in graft survival at 3, 6, and 12 months after transplantation. For further statistical evaluations the chi-square test for the rxk table (for the 2x2-table with correction for continuity) and the Mann-Whitney U-test were used.

RESULTS

Two hundred and twenty-three patients were included in the study. There were 148 DRw6-negative recipients and 75 DRw6-positive recipients. Table II shows patient data and the main risk factors for graft survival in both groups. Significant differences were not found.

Table II. Patient data

	DRw6-negative patients (n=148)	DRw6-positive patients (n=75)
Male (%)	55	55
Mean age (years)	35	35
Mean match grade DR	0.80	0.75
Mean match grade HLA-AB	2.1	2.2
First transplantation (%)	80.5	85.4
Mean number of blood transfusions	4	6
Mean dialysis period (months)	24	26

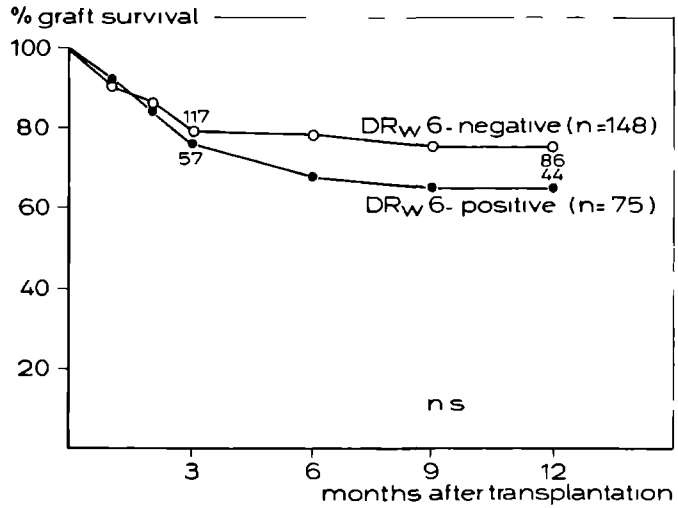

Fig. 1 Actuarial survival curves of cadaveric renal allografts in DRw6-positive and DRw6-negative recipients. Numbers of patients at risk are given on the curves (N.S. = not significant)

Actuarial survival curves for both groups did not differ significantly (Fig. 1). Graft survival at three months was 79% for the DRw6-negative recipients and 76% for the positive patients. Neither in the DRw6-negative nor in the DRw6-positive patients was a beneficial effect of HLA-DR matching found. Significant differences in patient survival between both groups were also not found. Patient survival after three months was 94% both for the DRw6-negative and positive patients. Detailed analysis of the post-transplant course in both patient groups failed to show significant differences for all but one variable (Table III). The percentage of patients without a rejection in the first three months after transplantation was significantly higher in the DRw6-negative recipients (41.2% vs. 25.3%; P=0.03). We, therefore, analyzed whether the form of treatment given for these rejections had influenced graft survival. Patients without a rejection in the first three months were excluded from this analysis. In the DRw6-positive group a highly significant influence was observed. When the first rejection had been treated with prednisone, graft survival after three months was 61%, while graft survival for the RATG treated patients was 88% (Fig.2; P=0.003). In the DRw6-negative patients graft survivals after three

Table III. Patient course in the first three months after transplantation

	DRw6-negative patients (n=148)	DRw6-positive patients (n=75)
Acute tubular necrosis (%)	43	43
Rejection free interval (days)	19.7	20.1
Urinary tract infections (mean per patient)	1.4	1.5
Respiratory tract infections (mean per patient)	0.3	0.2
Cytomegalovirus infections (%)	20	25
Herpes infections (%)	9	11
Mean prednisone dose (mg)	3400	3500
Mean serum creatinine (μmol/)	106	115
Patients without rejection (%)	41.2	25.3*

* P = 0.03. All other differences were not significant

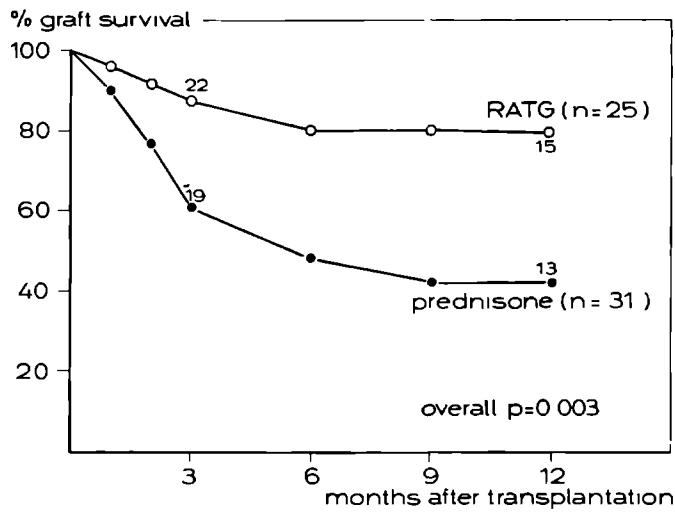

Fig. 2 Actuarial survival curves of cadaveric renal allografts in DRw6-positive recipients in relation to treatment for acute rejection. Patients without rejection in the first three months were excluded from the analysis

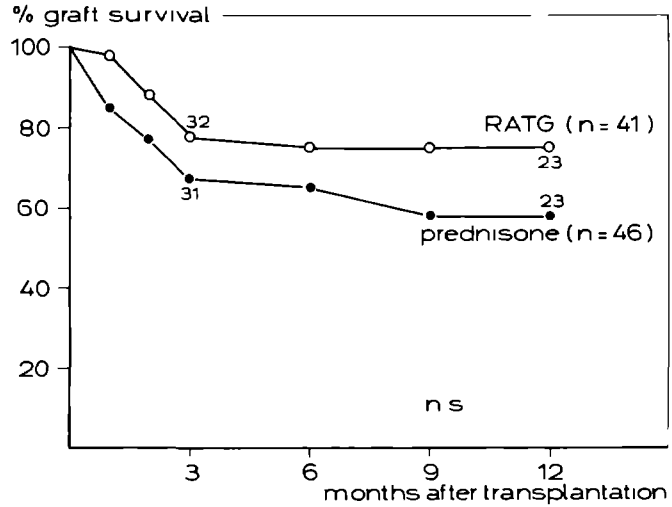

Fig. 3 Actuarial survival curves of cadaveric renal allografts in DRw6-negative recipients in relation to treatment for acute rejection (N.S. = not significant). Patients without rejection in the first three months were excluded from the analysis

months for patients treated with prednisone or RATG were 67% and 78% respectively (Fig.3), a difference that is not significant. In an attempt to obtain information on a group of patients, that resembled the patients studied by Hendriks et al[3] as closely as possible, we carried out an overall analysis of the whole group, including both patients with and without rejections in the first three months, but omitting all patients that had received RATG. A summary of all patient groups studied is given in Table IV. In this analysis a significant difference in graft survival between DRw6-negative and DRw6-positive recipients be-

Table IV. Summary of patient groups

	DRw6 +	DRw6 -
1. Total group (Fig.1)	75	148
2. RATG group	25 (Fig.2)	41 (Fig.3)
3. Prednisone group	31	46
4. Patients without rejection	19	61
5. Total of groups 3 and 4 (Fig. 4)	50	107

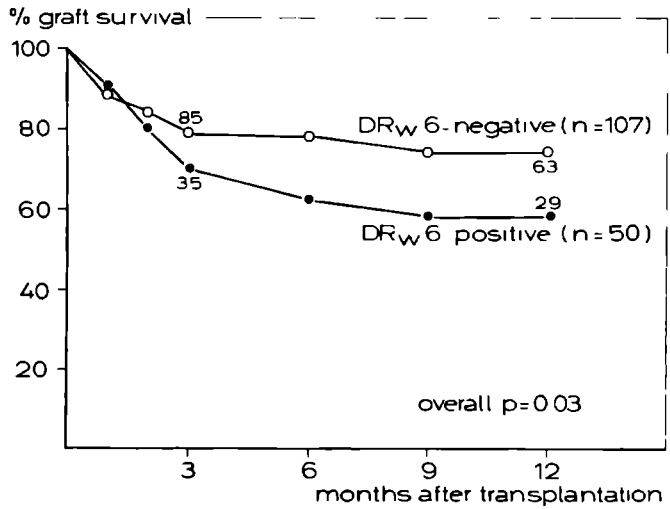

Fig. 4 Actuarial survival curves of cadaveric renal allografts in DRw6-positive and DRw6-negative recipients with exclusion of patients who received RATG as a treatment for their first rejection

came apparent (Fig. 4, P=0.03). Also in this subgroup we found no significant influence of DR matching on graft survival, neither in the whole group nor in the DRw6-positive and negative groups separately. Also when donor-patient combinations in which only one HLA-DR specificity of the donor was known were omitted from this latter analysis, a significant influence of DR matching was absent in either group. In all analyzed subgroups, patients who had received a kidney with one DR mismatch performed slightly better than DR matched combinations.

DISCUSSION

The higher percentage of rejections in the recipients who carried the DRw6-antigen suggested that these patients had an increased immune responsiveness to the graft. This is in accordance with the data of other groups[3,4,8]. However, in contradistinction to these reports this higher incidence of rejections was not reflected in a decreased graft survival. We supposed that this might be related to the fact that a large

group of our patients had received RATG as an antirejection treatment. Since April 1979 we had carried out a randomized trial in which patients who developed an acute rejection of their graft were treated with either RATG or high oral doses of prednisone[5,6]. A second rejection was treated with the alternative protocol. The results of this trial demonstrated that the treatment chosen for the first rejection episode was of importance. Patients who received RATG as the first treatment had a significantly better graft survival than those who received initial prednisone treatment (in preparation). Our current analysis shows that this beneficial effect of RATG was especially apparent in the DRw6 positive group, where graft survival after RATG was 88% at 3 months and 80% at one year. Graft survival rate in prednisone-treated DRw6 positive patients was significantly worse (61% and 42% at 3 and 12 months respectively). If patients who received RATG for their first rejection were eliminated from the overall analysis, a significant difference of graft survival between DRw6 positive and negative recipients became detectable. These results suggest that treatment with RATG for first rejection episodes had obscured the deleterious effect of the presence of DRw6 in the recipients. A recent report[8] in which administration of antilymphocyte globulin did not improve the survival in DRw6 positive recipients, seems to contradict our findings. However, the study comprised only six DRw6-positive patients, who received the antilymphocyte globulin. Furthermore, the preparation used was from a different source and was given as a continuous treatment from the day of transplantation, whereas in our study RATG was started only after the diagnosis of rejection had been made. These differences might explain the failure of others to find a beneficial effect of antilymphocyte globulin in DRw6-positive recipients.

We observed no influence of DR matching on graft survival rate in our patients, neither for the whole group nor for the DRw6-positive or DRw6-negative recipients. Such an influence was also absent when patients who received RATG-treatment were omitted from the analysis. We actually found in all analyses that patients who received a 1-DR-mismatched graft consistently showed a better graft survival than patients with a DR-matched or a 2-DR mismatched graft. The differences were, however, not significant. In this regard we could not confirm the fin-

dings of Hendriks et al[3] who found that matching for DR antigens is of great importance in DRw6-positive recipients. There is, however,another study in which an influence of DR-matching in these patients is also absent[8]. More data are obviously required to settle this point.

Our results suggest that, especially in DRw6-positive recipients, first rejections should be treated with antithymocyte globulin rather than with high doses of prednisone.

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CHAPTER 6

IMPROVED PATIENT AND GRAFT SURVIVAL AFTER TREATMENT OF ACUTE REJECTIONS OF CADAVERIC RENAL ALLOGRAFTS WITH RABBIT ANTITHYMOCYTE GLOBULIN

Andries J Hoitsma, Henk JJ van Lier, Paul Reekers, and Robert AP Koene

ABSTRACT

In a prospective randomized trial, we compared the effectiveness of rabbit antithymocyte globulin (RATG) in the treatment of acute renal allograft rejection with the results of treatment by high oral doses of prednisone. Fifty recipients of cadaveric kidneys were included in each group. In the RATG group, the prednisone dose was not increased and a dose-by-rosette protocol was used to keep T cell levels between 50 and 150/mm^3. The three months and one year graft survival rates in the RATG group were 84% and 78%, and were significantly higher than those in the Prednisone group (64% and 50%). A significant difference in patient survival could also be detected. In the RATG group the three months and one year patient survival rates were 100% and 98% , while patient survival rates in the Prednisone group were 91% and 84% respectively. The percentage of second rejections was higher in the Prednisone group and 70% of these patients showed a good response to subsequent RATG treatment. Renal function after six months was similar in both groups. No serious side effects were encountered in the RATG group. The incidence of infections was the same in both groups. Treatment of acute rejections with RATG is preferable to prednisone treatment. It improves long term graft and patient survival and is steroid sparing.

INTRODUCTION

Although heterologous antilymphoid globulin (ALG) has proven to be a highly effective immunosuppressive agent in animal experiments the question of whether treatment with ALG improves renal graft survival in man is still a matter of debate. Reports on the prophylactic use of ALG during the first weeks after transplantation are contradictory. In recent years several studies have appeared that suggest a greater efficacy of ALG when used not prophylactically, but as an agent to treat already established rejections[1-13]. Interpretation of the results of these studies is hampered by the fact that in many protocols ALG was used as an adjunct to treatment with high doses of steroids[1-5,7-10]. Until now only two controlled studies have been published in which ALG was the sole drug to treat a rejection crisis, and in both a good response was demonstrated[6,13]. Again, the results are difficult to interpret because one study concerned only transplants from living related donors[6], and in the other the procotol included also prophylactic treatment with ALG[13]. We have previously reported on our preliminary results of a randomized controlled trial in which rabbit antithymocyte globulin (RATG), used as the sole treatment in acute rejection of cadaveric renal grafts, was compared with prednisone treatment[11]. The present report deals with the final outcome of this trial.

PATIENTS AND METHODS

Only patients with first rejection episodes within three months after transplantation were admitted to the protocol. Related donors, diabetic patients, and patients who had received a RATG course earlier were excluded. The prospective trial included patients transplanted between February 1979 and December 1982. The trial ended in July 1983, when all patients had been followed for at least six months after transplantation. During the period of the trial 227 transplantations were carried out in our hospital. Included in our trial were 100 recipients of a cadaveric renal allograft of which 50 received high oral doses of prednisone and 50 were treated with RATG for their first acute rejection.

Sixty-six patients who did not experience a rejection in the first three months after transplantation served as a control group. Sixty-one recipients were excluded for the reasons given in Figure 1. The RATG we used was prepared according to the method of Kreeftenberg[14]. Patients were allocated to the different treatment groups initially according to the minimization method of Taves[15], later on according to the standardized variance method[16,17], while balancing for the following risk factors: sex, age, blood group of the recipient, number of blood transfusions, number of mismatches for HLA-A, B, and DR antigens, presence of lymphocytotoxic antibodies and B cell antibodies, number of previous transplants, and time of onset of the acute rejection after transplantation. The treatment protocol has been described earlier[11]. Briefly, until the diagnosis of acute rejection was made all patients were treated similarly with prednisone and azathioprine. In the RATG group the acute rejection was treated with a three-week course of RATG intravenously. The initial dose was 4 mg/kg and further doses ranged from 2-7 mg/kg depending on the peripheral T-cell level, which we tried to

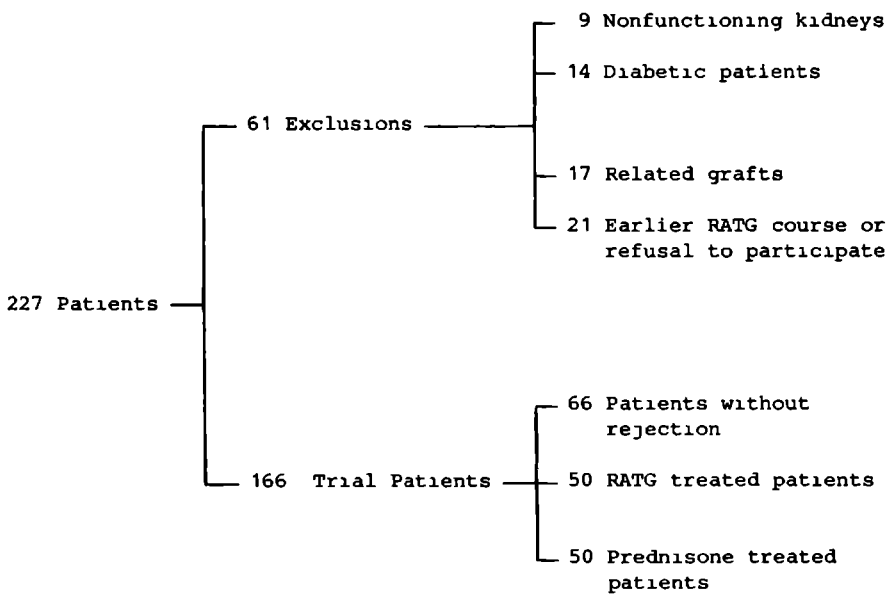

Fig. 1 All patients transplanted between February 1979 and December 1982 and the distribution in trial patients and exclusions

keep between 50-150 mm/^{3} [18]. The prednisone dose was not raised in this group. In the Prednisone group the oral dose of prednisone was raised to 200 mg/day and tapered in about two weeks to 25 mg. The alternative regimen was chosen if there was no improvement of renal function within 10 days. Second rejections were also treated with the alternative protocol. The day of reversal of an acute rejection was defined as the second of three consecutive days that the serum creatinine had decreased. The match grade for A-, B-antigens was calculated as follows: 1 point with 1 mismatch on the A-locus, 2 points with one mismatch on the B-locus, and 3 points with 2 or more mismatches. For the calculation of the DR-match grade one point was assigned for each DR-mismatch. Peripheral T cells were determined with the amino-ethylisothiouronium bromide (AET) rosetting technique[19]. Thrice weekly, RATG levels were determined with a radial immunodiffusion technique and antibodies to RATG with an agglutination assay. Antibodies to cytomegalovirus (CMV) were determined with an indirect enzyme-linked immunosorbent assay. Patients were considered to have had a prior CMV infection when the antibody titre, was higher than 1:2 and were considered to have a current CMV infection when clinical signs of CMV infection were accompanied by a fourfold increase in antibody titre. Graft failure was defined as a return of the patient to haemodialysis, regardless of whether the cause of failure was immunological or non-immunological. Patients who died, were also counted as graft failures, even if the graft was functioning at the time of death. Patient failures include all patients who died even if the cause of death was unrelated to the transplantation. For statistical evaluation the following methods were used: Mann-Whitney U test, test of Kruskal-Wallis, the Wilcoxon matched-pairs test, chi-square test. Survival at 3, 6, and 12 months were evaluated with the log rank test according to Mantel[20]. Survival rates were estimated with the survival procedure of the SPSS package. Differences were considered significant if P levels were less than 0.05.

RESULTS

Factors that might influence graft survival were similar in the RATG

and Prednisone group as shown in Table I. No significant differences could be detected. In Fig. 2 the graft and patient survival curves of all 166 patients are shown. The one year graft and patient survival were 76% and 96% respectively. The actuarial graft survival curves for both treatment groups are shown in Fig. 3. A significant difference in favour of the RATG treated patients was found (P=0.002). The three months graft survival was 84% and 64% in respectively RATG and prednisone treated patients. Included in the RATG graft failures is one patient who lost her kidney due to a technical failure. A significant difference in patient survival could also be detected (Fig. 4, P=0.02). The three months patient survival was 100% and 91% respectively in the RATG and prednisone treated patients. Details of the response to antirejection treatment in the two groups are shown in Table II. The results suggest that patients treated with RATG fared better with regard to nearly each variable, but none of the differences reached the level of significance. The number of patients in whom graft failure occurred so fast that a second antirejection treatment could not be given was four times higher in the Prednisone group (12 vs 3 patients,

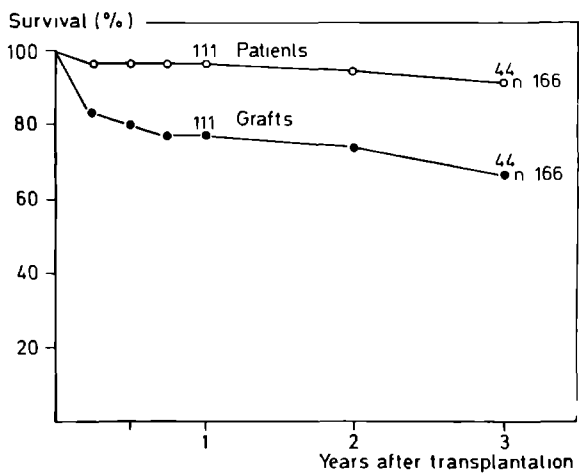

Fig. 2 Actuarial patient and graft survival curves of all 166 patients included in the trial

Table I. Comparison of possible risk factors

	RATG (n=50)	Prednisone (n=50)
Sex: male/female	27/23	28/22
Mean age (years) ± S.D.	35.5 ± 12.3	33.9 ± 12.8
Blood group: O/non O	20/30	14/36
Blood transfusions: one/two or more	19/31	26/24
Mean match grade		
AB-antigens	2.1	2.2
DR-antigens	0.7	0.7
DRw6-positive/negative	20/30	19/31
HLA antibodies		
none	31	28
⩽ 50%	8	11
> 50%	11	11
B cell antibodies		
negative	38	38
positive	9	7
unknown	3	5
Percentage of first transplantations	88	86
Interval from transplantation to rejection: ⩽12/>12 days	23/27	24/26

S.D. = Standard Deviation

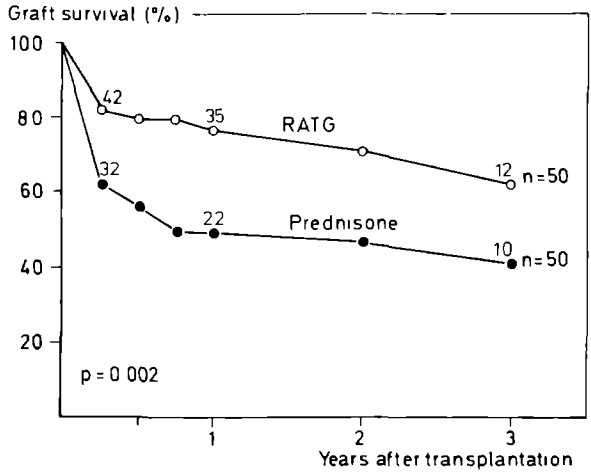

Fig. 3 Actuarial graft survival curves of the RATG group and the Prednisone group

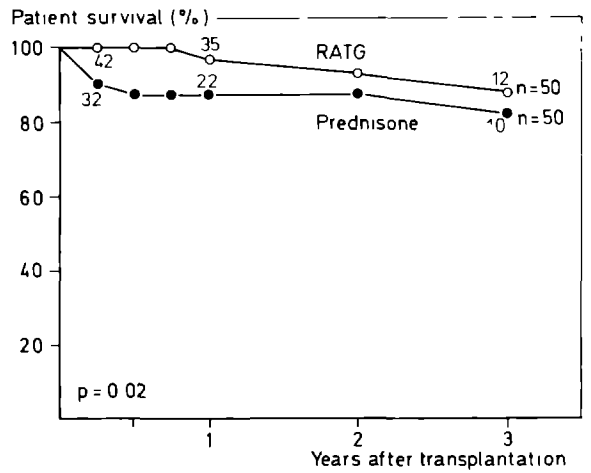

Fig. 4 Actuarial patient survival curves of the RATG group and the Prednisone group

P=0.02). In 20 patients of the Prednisone group a subsequent RATG treatment was given within three months either because of resistance to the first treatment or for a second rejection. Seventy percent of the patients responded to this treatment. Similarly, 17 patients in the RATG group received a subsequent prednisone treatment and 65% of the patients responded.

Table II. Response to antirejection treatment

	RATG	Prednisone
No reversal (number of patients)	7	15
Mean day of reversal	3.5	4.0
Second rejection episode (%)*	56.3	71.1
Interval between first and second rejection (days)	78	67
Mean survival of failed grafts (months)	11.0	6.3
Mean survival of patient failures (months)	19.9	6.1

* Percentage of patients at risk

Peripheral T cell levels were measured in all patients. At the onset of rejection, mean T cell levels of both treatment groups were similar (RATG, 1000 $\pm$ 507; prednisone, 1067 $\pm$ 592/mm^3), and not significantly different from the level of the control group of 66 patients without a rejection (951 $\pm$ 408/mm^3). In the RATG group the mean T cell level fell significantly during treatment (251 $\pm$ 200/mm^3) whereas this was not the case in the prednisone-treated group (964 $\pm$ 920/mm^3). The mean total RATG dose administered during 3 weeks was 1563 $\pm$ 599 mg and the mean frequency of RATG gifts was 7.6 $\pm$ 2.5. Differences in elimination rate of RATG might reflect its in vivo activity and therefore we measured RATG levels in the serum. There were no differences between RATG levels in the serum of patients who responded without further rejections (mean = 133 $\pm$ 60 ng/ml), and in the patients who had a second rejection within three months after transplantation (mean = 145 $\pm$ 61 ng/ml). In only

one patient were we able to detect antibodies against rabbit immunoglobulins. These antibodies developed 1 week after completion of the RATG course and this patient ultimately lost her kidney. Eight patients received a second course of RATG when they developed a third or fourth rejection. This course was given 3 weeks to 9 months (mean 4 months) after the first RATG course. All patients showed a good response and no serious side effects were encountered.

In Table III the side effects of the RATG treatment are listed. The most frequent adverse reactions were chills and fever which occurred almost exclusively during the first infusion. In none of the patients had RATG treatment to be discontinued because of side effects. The most serious problems were encountered in patients with pre-existing overhydration. These patients developed dyspnea with bronchoconstriction during the first infusion. We now treat these patients with RATG dissolved in maximally 100 ml of saline instead of the usual 500 ml and this has eliminated these reactions. The infection rates in both treatment groups and in the control group are shown in Table IV. No significant differences could be detected except for the number of cytomegalovirus infections, that was significantly lower in the control group, compared with both the RATG and Prednisone group. Percentages of patients with CMV antibodies prior to transplantation were similar in both groups.

The follow-up data are listed in Table V. The only significant difference between the RATG and prednisone treated patients was in the mean cumulative steroid dose within three months after transplantation. In the control patients we found a significantly lower serum creatinine, both at three months and at the end of the trial (July 1, 1983). Osteo-

Table III. Side effects of RATG treatment

Chills	31/50
Fever	31/50
Arthralgia	5/50
Bronchoconstriction	5/50
Nausea	5/50
Back Pain	4/50
Thrombophlebitis	2/50
Urticaria	1/50

Table IV. Infections in the first three months after transplantation*

	RATG	Prednisone	Control
Urinary tract infections	1.2 ± 1.7	1.5 ± 1.7	1.1 ± 1.1
Respiratory infections	0.40 ± 0.69	0.32 ± 0.62	0.18 ± 0.46
Cytomegalovirus infection	0.32 ± 0.47	0.30 ± 0.46	0.10 ± 0.30+

* Means ± standard deviation
+ Significantly different (P=0.009)

necrosis was four times more frequent in the Prednisone group compared to the RATG group or the control group, but this was not significant.

DISCUSSION

The preliminary results of our trial already showed that acute rejection episodes of cadaveric kidney grafts can be reversed by treatment with rabbit antithymocyte globulin. Analysis of the results at completion of the trial now demonstrate that this is reflected in significantly better patient and graft survival rates than in patients in whom acute rejections are treated with high doses of prednisone. This was accomplished with lower overall doses of prednisone and without the occurrence of serious complications.

The design of the trial was such that patients with second rejections received the alternative treatment protocol. Thus, in 20 patients of the Prednisone group RATG was given for a second rejection and 70% showed a good response. However, this favourable effect did not annihilate the difference in graft survival between both treatment groups. This is obviously caused by the fact that in a significantly larger number of patients in the Prednisone group graft failure during the first rejection episode occurred so fast that the alternative treatment could not be given. Our results, therefore, lead to the conclusion that RATG is especially more effective than prednisone in the treatment of first rejections. A graft survival after three months of 64% in the Prednisone group may seem excessively low, but one should realize that this figure only concerns patients with acute rejections in the first

Table V. Follow-up data

	RATG	Prednisone	Control
Mean serum creatinine level (µmol/l)			
at 3 months	113 ± 38	127 ± 72	93 ± 25+
at end of trial	138 ± 90	134 ± 126	108 ± 30+
Mean number of antihypertensive drugs (per patient)			
at 3 months	0.50 ± 0.76	0.76 ± 1.05	0.46 ± 0.87
at end of trial	1.09 ± 1.16	1.19 ± 1.21	1.00 ± 1.25
Mean proteinuria (g/24 hr)			
at 3 months	0.6 ± 2.1	0.3 ± 0.8	0.5 ± 1.6
at end of trial	0.9 ± 1.4	1.5 ± 3.9	0.8 ± 2.6
Osteonecrosis (number of patients)	1	4	1
Mean cumulative steroid dose after three months (mg)	3329 ± 681	4250 ± 519*	2978 ± 176+

+ $p < 0.05$ (Control vs both treatment groups)
* $p < 0.05$ (RATG vs Prednisone group)

three months after transplantation. If patients without rejection (38% of the whole group) are included in the survival calculations to give an overall impression of the results patient and graft survival rates are much higher (Fig.2). One year graft survival of the whole group was 76%, whereas patient survival was 96%. On the other hand, these figures may give a somewhat optimistic impression of our overall results, because 9 patients with primary non-functioning kidneys after transplantation were automatically excluded from our trial since we wanted to study the treatment of rejection. If these patients are also included in the calculations of the overall data, one year graft survival of the whole group becomes 73%, whereas patient survival remains the same, 96%.

The use of RATG only when rejection occurs has an important advantage over prophylactic treatment, since the considerable number of patients who do not develop a rejection are not unnecessarily treated with this agent. We adjusted the RATG dose to the peripheral T cell level and the mean frequency of the gifts was 7.6 times. The value of monitoring T cell levels is still an open question, but it enabled us to demonstrate that results can be obtained with much lower doses, than administered in fixed dose regimens where the frequency of administration may be as high as 21 gifts[3,5,7,8,10,12,13]. Another matter of debate is how long ATG must be administered. Three studies report about 10 days treatment[5,12,13], two about a two week course[6,8], and three about a three week course[3,7,10]. Only Nowygrod et al[9] reported on a comparison between courses of 10 and 21 days duration. They found that survival rates were the same, but that in the 10 day course the number of second rejections was higher. This point needs further study, but it suggests that courses shorter than three weeks are worthwhile trying.

Several adverse reactions were seen during RATG treatment but most were mild. More than half of the patients had chills and fever, which occurred almost exclusively during the first infusion. Although we could not detect antibodies to RATG (except in one patient) a few patients had symptoms suggestive of serum sickness (arthralgia and urticaria). In no case had we to stop the RATG treatment course because of side effects. We administered second RATG treatment courses to 8 pa-

tients and all of these patients tolerated the treatment very well.

We found four times more osteonecrosis in the Prednisone group than in the RATG group, and this is probably a result of the significantly higher steroid dose in the first three months after transplantation. This difference is however somewhat hard to evaluate because the incidence of osteonecrosis decreased over the last years, probably due to better haemodialysis techniques and the administration of active vitamin D drugs, and due to lower overall doses of steroids in the Prednisone group, because second rejections were always treated with RATG in this group.

It should be noted that our conclusions only apply to rabbit-ATG, because there may be great differences between ATG-preparations of different species not only with regard to immunosuppressive activity, but also concerning side effects and formation of antibodies against the foreign protein. We have chosen a rabbit-ATG because there is evidence that this is more immunosuppressive than equine-ATG[21].

In the last few years most attention in renal transplantation has been focused on Cyclosporin as a new and potent immunosuppressive agent and this tends to obscure the favourable effects that can be obtained with older immunosuppressive drugs. A word of caution seems to be justified here especially because of the uncertainty with regard to the nephrotoxicity of Cyclosporin after prolonged use. The one year survival rate in the patients who received ATG for their first rejection compares favourably with the results reported in the recent update of the European Multi-Centre Cyclosporin trial[22], despite the fact that our group comprised only patients at increased risk, because patients with no rejection in the first three months were excluded. The results obtained in the Prednisone group in our trial are almost the same as the 1 year graft survival figure in the European control group (52%). Our data suggest therefore that treatment of acute rejections with RATG is a good alternative for Cyclosporin. Apart from lacking the untoward side effects of Cyclosporin it is also a less expensive treatment. It remains to be determined whether treatment with Cyclosporin combined with RATG for acute rejections can improve the results any further.

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CHAPTER 7

GENERAL DISCUSSION

Antilymphocyte serum (ALS) is without any doubt immunosuppressive in man. Proof for this has been derived from studies in human volunteers, who have been treated with ALS alone. During treatment delayed hypersensitivity was suppressed and the survival of skin grafts from random donors was prolonged[1,2]. ALS has also been shown to prolong the survival of skin allografts used to cover excised third degree burns resulting in improved patient survival[2]. However, the efficacy of ALS in organ transplantation is still not firmly established. This applies more to the adjunctive use of ALS from the day of transplantation than to the antirejection treatment with ALS. An overview of the litterature of adjunctive therapy and antirejection treatment in human renal transplantation and the place of our investigation in this field is given below. For the compilation of this data we have used the original publications, and comments made in several overview articles[3-25]. In addition, data about toxicity of ALS and the new developments regarding the use of monoclonal antibodies are given.

I. Prophylactic treatment with ALS

In Table I the centers are listed that have used ALS as an adjunctive, prophylactic treatment. Many articles report on the same patients. To avoid duplications we have listed the papers according to center and have combined those dealing with the same patients. An exception has been made if centers report about ALS treatment in patients who also participated in ALS multicenter trials[85,91,108,109], or if an article deals with the combined data of several centers[105]. The centers are arranged chronologically according to the first year of the ALS study. If a center has carried out several different ALS studies, these are listed on several places in Table I with different years. If more than one center has started its ALS study in a particular year, an alphabetical order is followed.

The first clinical trial of ALS in human renal transplantation was done by Starzl and co-workers in 1966[26], and since then more than 50 centers have reported on their results. The problems with the initial studies of ALS as adjunctive immunosuppressant were many. Usually there is a

Table I Prophylactic use of ALS in renal transplantation

Center	Year of Start	No.of Pat.	Duration of Treatment	Specifications of Study			References	Final Outcome
Denver	1966	214	4 months	Ca/R	-	-	26-33	Improved graft survival
Lyon	1966	177	6 months	Ca/R	-	-	34-39	Less acute rejections
Basel	1967	29	3 months	Ca	-	-	40	No effect
Boston (PBBH)	1967	58	variable	Ca/R	C	-	41,42	Less acute rejections
Cleveland	1967	53	4 months	Ca/R	-	-	43,44	No effect
Rochester	1967	36	3 weeks	Ca/R	-	-	45,46	Less severe rejections
Salt Lake City	1967	8	variable	Ca/R	-	-	47	Less acute rejection, less steroids
Auckland	1968	10	variable	Ca	-	-	48	Less acute rejections, less steroids
Birmingham (USA)	1968	50	4 weeks	Ca/R	-	-	49,50	No conclusions
Boston (MGH)	1968	60	variable	Ca/R	-	-	1,51	No effect
Cincinnati	1968	50	variable	Ca/R	C	-	52,53	Spleen ALS better than thymocyte ALS
Johannesburg	1968	19	4 weeks	Ca	-	-	54,55	Less acute rejections
Louvain	1968	?	variable	?	?	-	56	No effect
Minneapolis	1968	±284	2-3 weeks	Ca/R	-	-	57-63	Better graft survival, less rejections, less steroids
Paris	1968	31	variable	Ca/R	C	-	64	No effect
Sydney	1968	124	variable	Ca	C	-	65-69	Better graft surival, less rejections
Zürich	1968	51	6 weeks	Ca	-	-	70	Graft survival at 1 year = 76%
Boston (BUH)	1969	39	5 times	Ca	-	-	71-74	Less acute rejections
Michigan	1969	±124	variable	Ca/R	C	-	75,76	No effect

Table I (continued)

Center	Year of Start	No.of Pat.	Duration of Treatment	Specifications of Study			References	Final Outcome
Brussels	1970	87	variable	Ca	-	-	77	Comparison of different ALS-batches
Gothenburg	1970	30	variable	Ca	-	-	78,79	No effect
Multicenter USA	1971	20	16 weeks	Ca	C	MC	80	Longer ALS treatment gives better results
Stockholm	1971	144	3 weeks	Ca/R	-	-	81,82	No conclusions
Birmingham (UK)	1972	43	10 days	Ca	C	-	83	No effect
Multicenter Can.	1972	87	10 days	Ca	C	MC	84	Less acute rejections
Halifax	1972	20	10 days	Ca	C	-	85	Favourable effect
Los Angeles (LACU)	1972	26	2 weeks	Ca/R	C	-	86	No effect
Philadelphia (JMC)	1972	38	2 weeks	Ca	-	-	87	Less acute rejections
Toronto (TGH)	1972	19	2-4 weeks	Ca/R	C	-	88	Less acute rejections
Richmond	1972	35	?	Ca	-	-	89	No effect
Zürich	1972	93	4 weeks	Ca	-	-	90	No improvement
Boston (MGH)	1973	60	variable	Ca	C	-	2,91	Improved graft survival, less early rejections
Multicenter USA	1973	183	2 weeks	Ca/R	C	MC	92	No effect
New York	±1973	±100	21 doses	Ca/R	C	-	93-95	Improved graft survival, less rejections
Stockholm	1973	103	3 weeks	Ca	C	-	96,97	No effect
Multicenter UK	1974	86	10 days	Ca/R	C	MC	98	No effect
Oslo	1974	20	4 months	Ca	C	-	99,100	No effect
Rennes	±1974	21	4 months	Ca	C	-	101	Improved graft survival, less rejections, less steroids
Richmond	1974	71	5 days	Ca	C	-	89,102	High potency ALS improves graft survival

Table I (continued)

Center	Year of Start	No.of Pat.	Duration of Treatment	Specifications of Study			References	Final Outcome
Birmingham (USA)	±1975	26	4 weeks	Ca	C	-	103	Improved graft survival
Philadelphia(AEMC)	±1975	35	2-4 weeks	Ca/R	C	-	104	Improved graft survival, less rejections
Boston (all cent.)	1976	120	21 doses	Ca	-	-	105	Improved graft survival
Los Angeles (CHLA)	1976	28	2-4 weeks	Ca	C	-	106	No effect
Richmond	±1976	40	?	Ca	-	-	107	Improved graft survival
Paris	1977	24	2-4 weeks	Ca	C	-	108,109	Improved graft survival, less steroids
Portland	1977	35	2 weeks	Ca	-	-	110	Less acute rejections
Cleveland	1978	31	2 weeks	Ca	C	-	111	Improved graft survival, second rejections later
Montreal	1978	40	2-4 weeks	Ca	-	-	112	No effect
Oslo/Stockholm	1978	30	3 weeks	Ca	C	MC	113	No effect
Toronto (TWH)	1979	21	3 weeks	Ca	-	-	114	Improved graft survival
Stockholm	1980	23	2 weeks	Ca	-	-	115,116	No effect

Ca = Cadaver Transplantation
R = Related Transplantation
C = Controlled Trial
MC = Multicenter Trial

Abbrevations of centers:
AEMC, Albert Einstein Medical Center
BUH, Boston University Hospital
CHLA, Childrens Hospital of Los Angeles
JMC, Jefferson Medical College
LACU, Los Angeles County University
MGH, Massachusets General Hospital
PBBH, Peter Bent Brigham Hospital
TGH, Toronto General Hospital
TWH, Toronto Western Hospital

lack of adequate controls of transplanted patients who did not receive ALS. The control groups were retrospective, being those patients treated before the introduction of this agent. Although the results of the first studies with ALS suggested a beneficial effect, they are somewhat hard to interpret, because in most centers overall management of the transplanted patients improved during this initial period. The controlled trials in the early years are not appropriately stratified for factors that are now known to influence graft survival (age, blood transfusions, HLA-typing, etc.), and therefore, even the results of these trials are hard to evaluate. With these restrictions in mind it can be concluded that 28 centers reported of a beneficial effect of adjunctive, prophylactic ALS treatment, 19 found no effect and the results of four centers were not conclusive. The successful results obtained with ALS most often regarded the occurrence of less acute rejections within the first three months after transplantation or the use of lower amounts of steroids, but without a concomitant improvement of graft survival. Improved graft survival was only reported by 14 centers. When we only consider the controlled trials, 25 could be evaluated. Nearly all of these controlled trials were done after 1972. The first controlled trials were performed in Boston[41,42], and Sidney[65-69], but both studies used a variable treatment schedule. The Boston group evaluated the ALS in both cadaveric and living related transplantations, and the Sydney group used both horse and goat ALS. The two groups reported a favourable effect. Of the 25 centers with a controlled trial 10 observed no effect at all of the ALS administration. Moreover, several controlled and non-controlled studies reported on the use of ALS in both cadaveric and living related transplantations, which makes comparison between treatment groups and between different centers more difficult. When we confine our analysis to the controlled trials concerning cadaveric donors only, five out of a total of 15 found no effect. If we narrow the criteria further by only selecting trials including more than 50 patients treated with ALS, one out of five showed no effect.

Another problem in evaluating the efficacy of ALS as prophylactic agent was the variability in treatment schedules used. Moreover a lot of patients could not complete these schedules because of side effects, whereas several studies were temporarily interrupted, because of lack

of ALS supplies. In Table II the treatment schedules are given related to the final outcome of the trial. No definitely favourable schedule can be derived from this data. There were two centers[71-74,89,102], who reported a favourable outcome with only five dosages of ALS. Both used rabbit ALS, and this brings us to the question in which species to raise the ALS.

Table II. Treatment schedules with prophylactic ALS

Treatment Schedule	Number of Centers	Favourable Effect
0 - 1 week	2	2
1 - 2 weeks	10	5
2 - 3 weeks	13	8
> 3 weeks	12	7
Variable	14	7

Most centers used a horse ALS and about 50% reported a beneficial effect. The reports about prophylactic use of rabbit ALS[71-74,83,89,102,107,114-116], stem from six different studies. Four reported a beneficial effect, while in Stockholm[115,116] and in Birmingham (UK)[83], the results were disappointing. In the latter study ALS raised in cows and pigs was also used which makes it less well comparable to the others. Two centers used goat ALS[52,53,65-69] and both reported a beneficial effect. Again, there are problems with the interpretation because one center also used an equine ALS[65-69], and the other compared the efficacy of ALS raised against spleen or thymocyte lymphocytes[52,53].

One of the main problems of the use of ALS in human renal transplantation is the lack of a satisfactory in vitro test that can give a predictive index of the efficacy of the agent prior to its injection in man. Commercially available horse ALS can differ in potency from lot to lot. Rabbit ALS poses an extra problem because the preparation of large batches is very laborious. Therefore, different batches, that may vary in potency, have to be used even during a single trial. The reason that it is used despite this disadvantage is the evidence that the rabbit is

the best producer of ALS[117]. Consequently, the amounts of protein that have to be administered to achieve the immunosuppressive effect are much lower than with horse ALS.

There is more agreement on the route of administration of ALS. In the earlier reports the subcutaneous and intramuscular route were used, but this was very painful, especially with horse ALS of which large volumes (up to 20 ml) are necessary. The intravenous route is now used by nearly everyone. It is the most convenient route for the patient, provided that administration is not too rapid, because this may result in a hypotensive episode due to bradykinin release from the destroyed circulating lymphocytes. Several clinicians prefer to inject ALS into large veins via an indwelling central venous catheter, but this is nearly never necessary when rabbit ALS is used. Also the use of an arteriovenous fistula or shunt should be avoided. We used in nearly all patients peripheral veins, even the tiny ones in small children, and we rarely saw thrombophlebitis. Treatment with horse ALS requires about four times more IgG and probably this larger amount of foreign protein more often causes thrombophlebitis.

Definite conclusions about the efficacy of ALS as an adjunctive agent in human renal transplantation cannot be drawn. The fact that so many centers did not find any beneficial effect makes it highly doubtful whether the approach of administering ALS from the day of transplantation is helpful in improving the results of renal transplantation.

II. Antirejection treatment with ALS

In contrast to the conflicting data on the use of ALS as a prophylactic agent in human renal transplantation, it has proved to be very effective in most studies where it was used to treat acute rejection episodes. The administration of ALS only at the moment of an acute rejection has obvious advantages as compared to a protocol of prophylactic ALS treatment. A considerable number of patients never experience an acute rejection and do not need unnecessary treatment with the foreign protein. In our department about 35% of the patients with a cadaveric transplant never experience a rejection, but, it is impossible to identify these patients in advance. Use of ALS as antirejection treatment

is also preferable because treatment from the day of transplantation introduces the risk that the patient has already formed antibodies against the foreign protein when the acute rejection occurs, thus making its treatment less effective.

Treatment of acute rejections with ALS has incidentally been tried since the early days of the use of ALS in renal transplantation and several centers have reported their results[35,37,39,48,56,65-69,85,114,118]. Almost all of these centers used ALS also as a prophylactic agent and few patients were treated with ALS for an acute rejection with varying success. The centers that have performed more systematic investigations on the use of ALS as antirejection treatment are given in Table III. They are listed chronologically according to the year that the ALS treatment was started. Again, centers with the same starting date are listed alphabetically. Of the three centers that are listed on two lines[125,126,130-133,136-140], the first line represents all patients, while the second line gives data of another study in a subgroup of patients. There are only three studies in which no effect was seen. All other centers report some beneficial influence. This varied from fewer second rejections, decreased requirements of steroids, or faster reversal of the serum creatinine to improved graft survival. The final outcome of this form of treatment proved to be much more favourable than of the prophylactic use. As can be seen in Table III, there were 10 centers that carried out a controlled trial, but only three of those tried to determine the efficacy of ALS antirejection treatment without a concomitant increase of steroids[125,130-132,137]. The controlled study from Boston[125] concerned only the treatment of acute rejections in living related donors and it might be expected that these rejections are more easily reversed than those of cadaveric grafts. The group from Cleveland used ALS also as prophylactic immunosuppressive agent for 14 days, and this may obviously have interfered with the reaction to the subsequent antirejection course. Anyhow, this policy enabled them to use very low doses of steroids. The only prospective randomized trial in cadaveric transplantation with ALS as the sole antirejection treatment was performed by our group[130-132]. As described in the preceding chapters we observed not only an improved graft survival, but also a decrease in mortality, without a concomitant rise in side effects or

Table III Antirejection treatment with ALS

Center	Year of Start	No.of Pat.	Duration Treatment (days)	Steroid Resistant	Specifications of Study		Adjunctive	References	Final Outcome
Cambridge	1967	7	variable	N	Ca	-	Y	119	No effect
Cleveland	1967	29	18 inject.	N	Ca/R	-	Y	43,44	No effect
Odense	±1973	14	21	N	U	C	Y	120,121	Faster reversal of rejection
Minneapolis	1974	25	10	N	Ca/R	C	Y	122	Fewer second rejections
New York	1976	32	10-21	Y	Ca/R	-	Y	123,124	Improved graft survival
Boston	1977	49	14	N	Ca/R	-	N	125,126	Favourable
	1977	10	14	N	R	C	N	125	Less steroids
Indianapolis	1977	23	15	N	Ca	C	Y	127,128	Improved graft survival
Minneapolis	1977	33	10	N	Ca/R	C	Y	129	Improved graft survival
Nijmegen	1979	50	21	N	Ca	C	N	130-132	Improved graft/ patient survival
	1979	6	21	Y	Ca	-	N	133	Favourable
Washington DC	1979	83	24	Y	Ca/R	-	Y	134,135	Favourable

Table III (continued)

Center	Year of Start	No.of Pat.	Duration Treatment (days)	Steroid Resistant	Specifications of Study		Adjunctive	References	Final Outcome
Cleveland	1980	37	10	partly	Ca/R	-	N	136-140	Favourable, also prophylactic
	1980	11	10	N	Ca	C	N	137	Favourable, also prophylactic
Detroit	1980	30	10	N	Ca	-	N	141	Favourable, also prophylactic
Frankfurt/Main	1980	24	10	Y	Ca	-	Y	142	Favourable (also plasmapheresis)
Madison	1980	35	14	Y	Ca	C	both	143	No improvement
Washington DC	1980	29	10	Y	Ca	C	Y	135	Favourable, also prophylactic
Philadelphia	1982	8	21	N	Ca/R	C	Y	144	Improved graft survival

Y = Yes
N = No
Ca = Cadaver Transplantation
R = Related Transplantation
C = Controlled Trial
U = Unknown

infections. In our study the assignment of the patients to the different treatment groups was obtained with an allocation method by which balance was achieved in advance between factors that are known to influence graft outcome[145]. This method is relatively time consuming but it has the important advantage that there is no imbalance in important risk factors between both groups at the end of the trial. A problem that can always disarrange the balanced randomization is the identification of new important risk factors. In the course of our trial we encountered this difficulty, when the role of the DRw6 antigen became apparent[146]. When we analyzed the influence of the HLA-DRw6 antigen on graft survival in our patients we initially did not find an influence of DRw6. However, on more detailed analysis we could demonstrate that the deleterious influence of the HLA-DRw6 antigen was counterbalanced by the antirejection treatment with ALS[147-149]. ALS antirejection treatment proved to be particularly favourable in DRw6-positive recipients. Although this factor was not taken into account during the trial, the distribution in the groups turned out to be equal at the end of the trial.

Only two centers reported on treatment with ALS raised in rabbits[130-133,142]. All the others have used equine ALS. The group from Frankfurt used ALS in steroid resistant rejections and, if in the biopsy a vascular rejection was found, ALS was combined with plasmapheresis, which leaves our study as the only one in which rabbit ALS was systematically used as the sole treatment for acute rejection. Thus, the available data does not allow a comparison between rabbit and horse ALS.

The two most frequently used treatment schedules are injections during 10 and during 21 days. There is no indication which schedule should be preferred. Only the group from New York[124] reported on a comparison between the two treatment schedules and found that graft survival was the same, but that recurrence of rejection was more frequent in the 10 day schedule. Although we have used a 3 week course during the trial we have now switched to a 10 day course and our early results are similar to our previous experience.

Another matter of debate is the adjustment of the ALS dose to periphe-

ral T cell levels. We tried to keep the T cells between 50 and 150/mm^3 to avoid excessive immunosuppression on the one hand or undertreatment of the acute rejection and occurrence of second rejection on the other. In only one other study a dose-by-rosette protocol was used[126]. In studies with fixed dose regimens the incidence of infections does not seem to be increased. Moreover, in several patients we could not suppress the T cells below 150/mm^3, despite daily injections, while the rejections in these patients nevertheless showed a good response to the treatment. Therefore, a dose-by-rosette protocol is probably not essential for optimal treatment of the patients.

One of the most striking examples of the efficacy of ALS as antirejection treatment is its use in rejections that do not respond to the conventional treatment with steroids. With the administration of ALS in these patients a lot of kidneys can be saved, which otherwise would have been rejected. Five centers have reported on this subject and all have found a favourable response[123,124,133-140,142,143]. Included is our limited experience with six steroid resistant rejections that all responded to additional ALS treatment. All centers report that ALS is still effective if used in second and further rejections. It is also important to note that a second or even third course of ALS can be given to the same patient without the occurrence of serious side effects and often with satisfactory response. The results of ALS treatment in late rejections, in which vascular damage mostly predominates, are hard to evaluate because of the diversity of clinical pictures. Our experience in these situations is not very encouraging[133]. The unanimity in the litterature about the effectiveness of ALS as an agent to reverse acute rejections and our findings that it improves patient and graft survival lead to the conclusion that it should only be used in this way after renal transplantation. Use of ALS as a prophylactic treatment is no longer warranted.

III. Toxicity of ALS

The fears that ALS treatment would be accompanied by severe side effects such as anaphylactic reactions or opportunistic infections have not come true, and surprisingly few side effects have been described. In Table IV a summary is given of the side effects mentioned in the

studies listed in Tables I and III and in a few other studies that mainly deal with adverse reactions to ALS[150-170].

Many studies do not give any details on the toxicity of ALS or indicate that there were no serious adverse reactions. Moreover, many of the early complications of ALS therapy were due to the contamination with antibodies to erythrocytes, platelets, serum proteins, or collagen, but with a better antigen choice and better absorption techniques a lot of these side effects, such as anemia, thrombocytopenia, and glomerulonephritis due to ALS deposits, have disappeared or have become rare events. In our series a moderate degree of thrombocytopenia was found in some patients, but none of the patients developed anemia or glomerulonephritis. Symptoms related to the injection site, e.g. pain, erythema, swelling, and even development of reticulosarcoma at the injection site, have disappeared since almost all centers have started to use the intravenous route. The incidence of thrombophlebitis has remained low even in our center where we often give the infusions into tiny veins as mentioned before. The most commonly observed side effect is a systemic reaction consisting of chills and fever. The incidence varies from 20-100%. It occurs almost invariably during the first infusion, but sometimes it recurs with each following infusion or it appears later in the course of therapy after many previous uneventful infusions. The chills and fever are possibly the result of the massive intravascular lysis of circulating lymphocytes. The fever is usually not high, and it is easily controlled by temporary slowing of the infusion rate combined with antipyretics and antihistamine drugs. The management of the patient during the ALS infusion has been described elsewhere[171,172]. It is most important to inform the patient in advance that these side effects can be troublesome but are relatively innocuous. Other systemic reactions are less common. Arthralgias, possibly a manifestation of serum sickness, occasionally occur, but in our and others' experience this does not necessitate termination of the ALS therapy. Very serious symptoms like anaphylactic shock have been rarely reported, but in none of these cases the anaphylaxis was convincingly proved. In the more than 100 ALS courses that we administered we have never seen an anaphylactic reaction. We encountered the most serious problems in patients with preexisting overhydration. These patients developed dyspnea with broncho-

constriction during the first infusion, and this has also repeatedly been reported in the litterature. We now treat these patients with RATG dissolved in maximally 100 ml saline solution instead of the usual 500 ml and this has eliminated these reactions.

Although these patients theoretically run an increased risk due to stronger immunosuppression achieved by ALS, a significant increase in bacterial sepsis associated with ALS therapy has not been reported. There are however several reports of an increased incidence of cytomegalovirus (CMV) infection, Epstein-Barr virus excretions, fungal infections, and herpes simplex infections during ALS treatment. It should be born in mind, however, that in nearly none of the patients ALS was given as the sole drug, and therefore many side effects observed in patients might be due to the other immunosuppressive agents or to the combination of the agents with ALS. An argument for this is the observation of Rubin et al[173], who found that the incidence of CMV infections did not increase in ALS treated patients, if the concomitant conventional therapy had been halved. Our own observation that the incidence of CMV infections was not increased, when ALS is given as the sole drug for rejection, fits with this concept[132].

Early studies also made mention of deposits of ALS, particularly horse ALS, in the kidney and in some cases this gave rise to the development of a nephrotic syndrome. This complication was probably caused by antibodies against basement membranes that contaminated the early preparations. The deposits of ALS never led to serious impairment of renal function, and with better purification procedures of ALS this side effect has disappeared. The incidence of neoplasms is approximately 80 times higher in renal transplant patients than in the average population[174]. This is probably related to the state of immunosuppression. One of the agents implicated was ALS[40,161,162], and its role is clear with regard to the development of reticulosarcomas at the site of injection, when ALS was given intramuscularly[44,159,160]. It was also thought to cause lymphoid hyperplasia[161,163,164]. Later analyses by Penn et al could not substantiate the suspicion that ALS plays a major role in the genesis of neoplasms[174-176]. In the five years that we had used ALS none of our patients had developed a tumor, while the overall

Table IV Complications of ALS treatment

Side effects	References
Hematologic Complications	
Anemia	150
Thrombocytopenia	2,37,47,48,63,65,66,69,70,78-82,84,86,92,93,96,97,110,111,119,126,141,142,150
Infectious Complications	
Cytomegalovirus infections	114,151,153
Epstein-Barr virus excretion	125,154
Fungal infections	155-157
Herpes simplex infections	126,144,151
Local Reactions at Injection Site	
Infection	35,47
Pain, swelling, erythema	1,27,33,34,36,39,43,44,47-50,64-66,69,73,75,92,101,115,158
Phlebitis	2,37,39,81,91,92,96-98,112,113,130-132,142
Oncogenic Complications	
Local tumor growth	44,159,160
Malignant lymphoma	40,161,162
Pseudolymphoma	161,163,164
Renal Complications	
Deposits of ALS	70,150,165-167
Nephrotic syndrome	84,168

Table IV Complications of ALS treatment

Side effects	References
Hematologic Complications	
Anemia	150
Thrombocytopenia	2,37,47,48,63,65,66,69,70,78-82,84,86,92,93,96,97,110,111,119,125,141,142,150
Infectious Complications	
Cytomegalovirus infections	114,151,153
Epstein-Barr virus excretion	125,154
Fungal infections	155-157
Herpes simplex infections	126,144,151
Local Reactions at Injection Site	
Infection	35,47
Pain, swelling, erythema	1,27,33,34.36,39,43,44,47-50,64-66,69,73,75,92,101,115,158
Phlebitis	2,37,39,81.91,92,96-98,112,113,130-132,142
Oncogenic Complications	
Local tumor growth	44,159,160
Malignant lymphoma	40,161.162
Pseudolymphoma	161,163,164
Renal Complications	
Deposits of ALS	70,150,165-167
Nephrotic syndrome	84,168

Table IV (continued)

Side effects	References
Systemic Reactions	
Anaphylaxis	27,33,37,39,42,44,115,127,128, 158
Arthralgia	37,39,66,67,75,80,91,92,109, 125,130-132,141,142,158
Chills	1,2,26,27,34,36,37,39,48,49,63, 70,78-81,84,92,93,109,111,115, 118,125,130-132,141,158,169
Dyspnea	37,39,48,66,75,80,98,113,130-132
Epidermal necrolysis	109
Fever	1,2,26,27,34,36,37,39,47-50,53, 57,63,64,67,69,70,73,75,78-82, 84,86,91-93,101,109,111,113, 115,118,125-128,130-133,135, 136,141,142,158,169
Hypotension	26,34,36,43,44,48,86,92,115
Lumbar pain	37,39,57,81,92,130-132
Periorbital edema	1,81,158
Pruritus	2,37,39,43,44,80,82,91,92,111, 158
Rash	2,36,37,39,47,57,78,81,82,91, 92,111,158,169
Serum sickness	1,2,4,36,53,61,75,78,84,91,130-133,170
Tachycardia	36,92,100,113
Urticaria	1,27,36,43,45,46,50,57,59,63, 66,75,79,82,84,91,92,96,97, 100,113,130-132,158
Vomiting	[illegible]

incidence of tumors in our renal transplantation programme is 2.8%[177].

In summary, we may conclude that severe toxicity from ALS treatment has not been reported. A lot of the earlier described complications have disappeared. We have not been forced to discontinue a single ALS course because of the side effects. The safety of ALS treatment is once more confirmed by our finding that repeated ALS courses could be given without major adverse reactions.

IV. Monoclonal antithymocyte globulins

Although ALS has proved to be highly efficacious in reversing an acute rejection in renal transplantation, the agent has some drawbacks. ALS not only contains a heterogeneous group of antibodies to human T cells, but also a high amount of other irrelevant antibodies. In comparison to the less specific immunosuppressive agents like azathioprine and prednisone humoral immunity is probably less impaired, but the patient under ALS treatment is still vulnerable to viral and fungal infections. Furthermore, the potency of the ALS can vary from batch to batch and a consistent product cannot be guaranteed. Therefore, the search for more specific agents has continued. Thanks to the development of the hybridoma technique[178] monoclonal antibodies to human T cells can now be produced. The preparations are completely standardized and not contaminated by irrelevant antibodies. The purpose of this section is to give a short overview of the in vitro and in vivo results obtained with monoclonal anti T cell antibodies. Additional information can be found in some reviews on this subject[24,179-182].

In vitro results

It has been postulated that monitoring of the peripheral T cell levels might provide a reliable measure of the adequacy and safety of an immunosuppressive therapy. Many investigators have used monoclonal antibodies to monitor the peripheral T cell levels before and after renal transplantation and although some groups claimed that T cell levels,levels of T cell subsets, or T cell subset-ratios can be used to predict the occurrence of a rejection, most of the studies report rather conflicting data[183]. Some agreement has now been reached with regard to the relation of the T helper/T suppressor ratio to allograft function.

Patients with a high ratio were likely to suffer at least once from an acute rejection, but the ultimate outcome was excellent, while patients with a low ratio were less likely to have an acute rejection, but if it occurred, the ultimate outcome was bad. This rule is, however, not valid in patients treated with cyclosporin A. The T helper/T suppressor ratio is probably also indicative for the presence of CMV infection. These patients mostly have a very low ratio, and this might be useful to discriminate between CMV infection and rejection[183]. By monitoring T cell levels with the AET-rosette technique, we could neither predict an acute rejection nor the ultimate outcome of the graft. This is not so surprising, since the immune response is regulated by distinct T cell subsets and important changes in one or several subsets may not be reflected in the total T cell count. We have also performed some studies to investigate the role of the T cell subsets determined with the OKT monoclonal antibodies. The results were disappointing and we could not confirm the findings of other investigators. The number of lymphocytes obtained from heavily immunosuppressed patients, and particularly from ALS treated patients, were often very low, and the lymphocytes obtained from patients by separation techniques were mostly so heavily contaminated with non-lymphocytes that a proper determination of T cell subsets was impossible. In using whole blood samples of these patients we furthermore found that the red blood cells were highly resistant to lysis, so that purified leukocyte suspensions could not be obtained without gradient centrifugation. Many of the conflicting data reported in the litterature may be due to these technical difficulties. We found great day to day fluctuations in the values of the same patient. We were therefore not able to use the T cell subset levels to predict the occurrence of rejection or the outcome of the graft. Discrimination between CMV infection and rejection was also impossible in our hands.

In vivo studies

Before the monoclonal antibodies against T cells could be used in a clinical situation, an evaluation of their immunosuppressive potency and toxicity in animal studies was required. Because the monoclonal antibodies were so specifically directed against human T cells, only some reacted with T cells of non-human primates, and could be tested in these models. In Table V the studies in monkeys with monoclonal antibo-

dies against different human T cell populations are summarized. The first study was done by Cosimi et al[186], who tested an antibody against T helper cells in Cynomolgus monkeys carrying a renal allograft. Only a minute amount of antibody (17-51 mg/recipient) was necessary to obtain an improved graft survival. This beneficial effect was achieved without a concomitant decrease of the total T cells, while the helper T cells gradually decreased (from ± 800 to ± 250/mm^3). Bieber et al[184] found a profound decrease of T cells in three monkeys treated with a pan T cell antibody. One of the monkeys also received a skin graft, which showed a significantly prolonged survival. From Table V the main drawbacks of these monoclonals can also be seen. Although not all investigators reported on the incidence of antibody formation against mouse IgG, it was apparent that antibodies very often developed and this interfered with the effectiveness of the treatment. Other problems were the occurrence of T cell coating and antigenic modulation. Antibody-coating is a phenomenon in which cells have obviously bound the monoclonal antibody, but this does not result in clearance of these cells from the circulation. The most likely explanation is that the antibody does not lead to an effective opsonization of the coated cell due to its low density on the cell membrane or because it belongs to a subclass that cannot bind to the recipients opsonizing system. Antigenic modulation is a reversible loss of specific membrane antigens due to internalization or shedding of the antigen after binding of the monoclonal antibody. A few days after cessation of monoclonal antibody administration the specific membrane antigen towards the monoclonal antibody is directed will reappear. The inhibitory role of modulation and coating during monoclonal antibody therapy is not quite clear, since several investigators reported a beneficial effect despite the occurrence of modulation or coating[186,188], while in another report there was no improvement of graft survival when these phenomena were observed[187]. Generally, the animals did not show serious side effects, although Billing[185] reported on four subsequent lethal events, when he administered high intravenous doses of an anti-Ia-like antibody. The mechanism of this lethal effect is unknown, but is probably related to the specificity of this monoclonal antibody and not to an anaphylactic response to mouse protein, since treatment with smaller doses did not lead to serious problems.

Table V Preclinical use of monoclonal anti T-cell antibodies

Investigators	No.of Recipients	Monoclonal Antibody Against	Treatment Schedule (days)	Antibody Formation (No.)	Coating (No.)	Modulation (No.)	Final Outcome
Bieber et al[184]	3	Total T cells	1 dose	?	?	?	Profound fall of T cells
Billing et al[185]	9	Ia pos cells	14?	?	?	?	Improved skin graft survival
	6	Blast cells and monocytes	14?	?	?	?	Improved skin graft survival
Cosimi et al[186]	10	Helper T cells	7-14	?	+	?	Prolonged kidney graft survival
Giorgi et al[187]	3	Total T cells	12-14	3	+	+	No effect
Jonker et al[188]	6	Helper T cells	14	6	4	0	Improved skin graft survival
	4	Suppressor T cells	14	4	2	0	No effect
	4	Total T cells	14	4	0	4	Improved skin graft survival

(+); has been reported, but number of monkeys unknown

In summary, these animal studies indicate that monoclonal antibodies can be administered without serious side effects and that most of the monoclonals directed against T cells exerted an evident immunosuppressive effect.

Encouraged by the ease of administration, the effectiveness, and the lack of toxicity of monoclonal antibody therapy in nonhuman primates, a few centers started to evaluate the efficacy of monoclonal antibody in human renal transplantation (Table VI). Cosimi et al. were the first to report on a clinical trial with a monoclonal antibody against total T cells and they used it to treat acute rejections. Although they observed reversal of acute rejection in all cases, their results were somewhat disappointing, because almost all patients suffered from a subsequent rejection and developed antibodies against mouse IgG. In the other studies all but one[192] used a monoclonal against total T cells. Takahashi et al used an antiblast monoclonal antibody which was effective against proliferating T cells and did not depress T cell levels. Although this monoclonal reacted also with stem cells in bone-marrow, and with monocytes they found no serious side effects and could reverse acute rejections in 90 percent of the cases. Interestingly, the only monoclonal that did not produce modulation or antibody formation was not effective in reversing renal allograft rejection[193]. This antibody was of the IgG2b subclass. We suspect that this was the reason why it was ineffective because there is preliminary evidence from our laboratory that monocytes of almost all human individuals cannot bind the Fc fragment of mouse IgG2b resulting in lack of removal of antibody coated cells (W. Tax, personal communication).

Most of the antibody response of the recipient is probably not directed against allotypic determinants but against the idiotype of the monoclonal antibody. The development of antiidiotypic antibodies after monoclonal therapy was nicely demonstrated by Cosimi's group[194]. There is now evidence that repeated injections of monoclonal antibodies in the presence of circulating antiidiotypic antibodies do not lead to anaphylactic or other allergic reactions. The inhibitory effect of the antiidiotypic antibodies can therefore easily be overcome by administering higher doses of monoclonal antibodies (P. Capel, personal communica-

Table VI Clinical use of monoclonal anti T-cell antibodies in renal transplantation

Investigators	No.of Pat.	Treatment Schedule		Anti-Isotypic Antibodies	Anti-Idiotypic Antibodies	Antigenic Modulation	Final Outcome
		Mode	Duration (days)				
Chatenoud et al[189]	6	P	14	+	+	+	Profound decrease of T cells
Cosimi et al[190,191]	8	T	variable	+	?	+	Reversal of all rejections
Takahashi et al[192]	19	T	9	+	?	?	Beneficial effect
Thurlow et al[193]	3	T	10	-	-	-	No effect

P; Prophylactic, T; Therapeutic

tion). Modulation also occurs after injection of monoclonal antibodies in man. Until now, this has only been demonstrated after injection of OKT3 antibody[189-191,195]. The consequences of this phenomenon in the clinical situation are not yet clear.
Chills and fever invariably occurred following the first or second infusion of monoclonal antibody, but were usually not noted during subsequent infusions, suggesting rapid cell lysis as the etiology of this toxicity. Other side effects were not noted.

In summary, the results of monoclonal anti T cell antibody therapy in human renal transplantation are until now not very impressive. Most rejections could be treated, but rapid subsequent rejections and the development of antibodies pose an extra problem. Clinical trials with other monoclonal antibodies and particularly with combinations of monoclonal antibodies will give a more definite answer with regard to efficacy of monoclonal antibodies in renal transplantation. Monoclonal antibodies that are active against proliferating T cells such as the antibody described by Takahashi et al[192] are of special interest, because they will specifically eliminate those cells that are triggered by the MHC antigens of the donor. In our laboratory, Tax recently has developed an antibody (WT1) that is specifically reactive with all T cells. He has shown that the expression of the antigen against which it is directed shows a large increase on proliferating T cells[196,197]. The WT1 antibody has already been shown to have immunosuppressive activity since it can prolong skin graft survival in Rhesus monkeys[188]. The antibody is an obvious candidate for clinical application, and we are currently preparing it for a human pilot study, in which we will use it for the treatment of acute rejection.

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Niertransplantatie is een algemeen geaccepteerde vorm van behandeling van terminale nierinsufficientie geworden met grote kans op volledige revalidatie van de patient. De uremische patient, die aangewezen is op behandeling met chronische dialyse wordt niet alleen in zijn doen en laten beperkt door de steeds terugkerende dialysen, maar moet zich bovendien nog onderwerpen aan dieet- en vochtbeperking terwijl zijn algemene conditie desondanks meestal verre van optimaal blijft. De resultaten van niertransplantaties zijn na een aanvankelijke, sterke verbetering, de laatste jaren op hetzelfde niveau gebleven. De voornaamste oorzaak van een mislukte transplantatie is de onbehandelbare rejectie. Als immunosuppressieve middelen zijn door de jaren heen steeds prednison en azathioprine gebruikt, terwijl acute rejecties practisch altijd behandeld werden met verhogen van de steroid-dosis. Met deze middelen kunnen niet alle rejecties afdoende gecoupeerd worden, terwijl er anderzijds wel veel ernstige bijwerkingen optreden als gevolg van de algemene vermindering van de afweer. Om de resultaten van niertransplantaties te verbeteren zijn sterkere en liefst ook meer specifieke immunosuppressieve middelen nodig. De cel, die een centrale plaats inneemt bij een acute afstoting is de T-lymfocyt. Eliminatie van T cellen door antilymfocytenserum (ALS) zou, althans in theorie, een meer specifieke therapie kunnen zijn ter voorkoming en behandeling van acute rejecties.

ALS is al een oud middel. Vanaf het begin der zestiger jaren is uit veel dierproeven gebleken dat ALS de transplantaatoverleving kan verlengen. Vanaf 1967 is ALS klinisch gebruikt als immunosuppressief middel, in eerste instantie direct in aansluiting aan de transplantatie om het ontstaan van rejecties te voorkomen. Het effect van deze vorm van behandeling was minder gunstig dan men op grond van de resultaten verkregen in de dierproeven zou mogen verwachten. Bovendien werd beoordeling vaak bemoeilijkt, doordat adequate controlegroepen ontbraken. In de laatste jaren kwamen er uit enkele oriënterende studies aanwijzingen dat ALS mogelijk meer geschikt was voor de behandeling van manifeste rejecties dan voor de profylaxe ervan. Ook hier bleef het trekken van verantwoorde, definitieve conclusies onmogelijk door het ontbreken van gecontroleerde studies. In een poging de resultaten van de niertrans-

plantaties te verbeteren en tegelijkertijd de effectiviteit van ALS als antirejectiemiddel te testen, zijn we in 1979 gestart met een onderzoek, waarbij behandeling met ALS zonder verhoging van de steroïd-doseringwerd vergeleken met de conventionele antirejectiebehandeling bestaande uit hoge doseringen prednison. Een van de basis-eisen van het onderzoek was de aanwezigheid van een adequate controle-groep en daarom is vanaf het begin in overleg met de Mathematisch-Statistische Adviesafdeling (MSA) gestreefd naar een evenwichtige verdeling van de patiënten in de twee behandelingsgroepen. De patiënten werden gerandomiseerd zodra een acute rejectie was opgetreden waarbij rekening werd gehouden met alle risicofactoren, waarvan bekend is dat ze van belang zijn voor de transplantaatoverleving. Voor de factoren die al bekend waren op het moment van transplantatie was dat niet zo'n probleem, maar een drietal factoren kon pas vastgesteld worden op het moment dat de diagnose rejectie gesteld werd (interval tussen transplantatie en rejectie, tijdstip van laatste hemodialyse en lichaamstemperatuur van de patiënt). Aangezien de MSA in het algemeen niet onmiddellijk geconsulteerd kon worden, als een rejectie werd vastgesteld, moest voor een juiste randomizatie van de patiënten een nieuwe procedure ontwikkeld worden. Zo snel mogelijk na een transplantatie werden de bekende risicofactoren doorgegeven aan de MSA, die de patiënt randomiseerde voor die factoren en een achttal gesloten enveloppen plaatste op de verpleegafdeling met al de mogelijke combinaties van de nog niet bekende risicofactoren. Op het moment van rejectie werd dan de passende envelop geopend waarin de voorgeschreven antirejectiebehandeling stond vermeld. Op die - zij het nogal tijdrovende - manier waren we bij de start van het onderzoek al verzekerd van een juiste verdeling van de risicofactoren.

Toegelaten tot het onderzoek werden alleen patiënten die een cadavernier hadden ontvangen en een acute rejectie kregen binnen 3 maanden na niertransplantatie. Deze laatste voorwaarde was nodig aangezien alleen cellulaire rejecties behandeld zouden worden en de kans dat de rejectie vasculair is na drie maanden sterk toeneemt. De antistof die we gebruikt hebben, werd bereid in het Rijks Instituut voor de Volksgezondheid door immunisaties van konijnen met menselijke thymocyten. Via enkele zuiveringsstappen werd uit het serum van de geïmmuniseerde dieren de globuline fractie verkregen (ATG). Tijdens een antirejectiebehande-

ling werd het ATG intraveneus toegediend gedurende 3 weken, waarbij een zogenaamd "dose-by-rosette" protocol werd gebruikt. Bij een dergelijk protocol wordt getracht de perifere T cellen - bepaald met de rosette test - binnen bepaalde grenzen te houden door de ATG dosering aan te passen. De gehanteerde onder- en bovengrens voor de T-cellen waren respectievelijk 50 en 150 per mm^3. Beneden de ondergrens zouden meer infecties voorkomen door te sterke immunosuppressie, terwijl erboven de kans op rejectie zou toenemen. Tijdens ATG-therapie werd de prednisondosis niet verhoogd, om de schadelijke bijwerkingen van hoge doseringen prednison zoveel mogelijk te vermijden.

Na bijna vier jaar werd het onderzoek afgesloten toen 50 patienten met een acute rejectie behandeld waren met ATG en 50 patienten in de controlegroep met hoge doseringen prednison. De T cellen waren gedurende ATG-therapie in de meeste gevallen gemakkelijk binnen de gestelde grenzen te houden. In de ATG-groep werd dan ook een significante daling van de T cellen gevonden tijdens de antirejectietherapie, terwijl in de prednison-groep er geen verandering ontstond in het T-cel-niveau. De uiteindelijke transplantaat- en patientoverleving, bepaald een half jaar na het beëindigen van het onderzoek, was significant beter in de ATG-groep dan in de groep behandeld voor acute rejectie met hoge doseringen prednison. Het aantal tweede rejecties was lager in de ATG-groep. Als er een tweede rejectie in de ATG-groep optrad, dan gebeurde dat later dan bij de patienten uit de prednisongroep. Bovendien ontstond de daling van het serumkreatininegehalte sneller in de ATG-groep. Ook patienten met een tweede rejectie (overigens meestal behandeld buiten het protocol), bij wie de eerste rejectie behandeld was met hoge doseringen prednison, konden afdoende behandeld worden. Met name was ATG ook effectief bij de behandeling van de steroid-resistente rejecties. Dit waren de rejecties die in het geheel niet of zeer onvoldoende reageerden op conventionele therapie met steroiden. In alle gevallen kon het transplantaat behouden blijven na aanvullende ATG therapie. Minder effectief was ATG in de behandeling van vasculaire rejecties. Hoewel deze patienten (buiten protocol) aanzienlijk meer ATG kregen, lukte het niet om het aantal T cellen voldoende te doen dalen en was de reactie op de therapie voor wat betreft de nierfunctie eveneens erg matig. Overigens werden bij geen enkele ATG behandeling ernstige

bijwerkingen gevonden en in geen enkel geval moesten we de therapie staken wegens bijwerkingen. Infecties kwamen in beide groepen even vaak voor. De gemiddelde nierfunctie van de patienten met een nog functionerend transplantaat was gelijk in beide groepen. Uiteraard was de cumulatieve steroiddosering in de eerste drie maanden significant lager in de ATG-groep dan in de prednisongroep.Mogelijk als gevolg hiervan was het aantal gevallen van avasculaire botnecrose in de ATG-groep lager.

Een risicofactor die pas in de loop van het onderzoek duidelijk werd, was de aanwezigheid van het DRw6-antigeen bij de ontvanger. In enkele mededelingen in de literatuur werd er op gewezen dat DRw6-positieve ontvangers een significant slechtere transplantaatoverleving zouden hebben dan DRw6-negatieve patienten. Analyse van 223 transplantaatontvangers in ons centrum liet geen invloed van het DRw6-antigeen zien op de transplantaatoverleving, maar wel vonden we significant meer rejecties in de DRw6-positieve groep. Er was dus ook in onze populatie mogelijk een DRw6 effect, maar dit vond blijkbaar niet zijn neerslag in slechtere transplantaatoverleving. Het leek waarschijnlijk dat de aard van de antirejectie therapie hierbij van belang was. Inderdaad bleek het effect van antirejectie therapie met ATG significant beter dan dat van prednison in de DRw6-positieve patienten, terwijl in de DRw6-negatieve groep de verschillen veel minder uitgesproken waren. Wanneer de met ATG behandelde patienten buiten de analyse gehouden werden, dan werd de ongunstige invloed van het DRw6-antigeen op de transplantaatoverleving ook bij onze patienten zichtbaar. Omdat bij analyse achteraf bleek dat het DRw6-antigeen gelijk verdeeld was over beide behandelingsgroepen in het gerandomiseerde onderzoek leidde de identificatie van deze nieuwe risicofactor niet tot veranderingen in onze oorspronkelijke conclusies.

Antirejectiebehandeling met konijnen-ATG is dus superieur aan de conventionele therapie met hoge doseringen prednison. De transplantatieresultaten verbeteren sterk zonder dat deze blijkbaar krachtiger vorm van immunosuppressie leidt tot een vermindering van de afweer tegen infecties. Met name bij DRw6-positieve patienten is de ATG-behandeling effectief in vergelijking met prednisonverhoging. Ook tweede en steroidresistente rejecties kunnen uitstekend behandeld worden met ATG.

Allen, die hebben bijgedragen aan het tot stand komen van dit proefschrift, wil ik gaarne bedanken. In de allereerste plaats ben ik veel dank verschuldigd aan de patienten, zonder wier bereidwillige medewerking dit onderzoek onmogelijk zou zijn geweest. De arts-assistenten, die in de loop van dit onderzoek stage liepen op de afdeling nefrologie hebben het merendeel van de ATG infusen verzorgd en mede dank zij hun inzet zijn er tijdens de ATG kuren geen ernstige problemen opgetreden. Ook de verpleegkundigen van de verpleegafdeling nierziekten onder de bezielende leiding van Eugenie Burm hebben op nauwlettende wijze problemen tijdens de infusen voorkomen. Jos Paardekoper van het leukocytenlaboratorium heeft gedurende het gehele onderzoek practisch alle T cel bepalingen verricht. Differentiatie van vol bloed en celsuspensies met behulp van de Hemalog-D werd vakkundig uitgevoerd door Paul Ruys en Harrie Louwers van de afdeling hematologie. Jacqueline Hagemann en Peter Faaber namen de vele werkzaamheden rond de bepaling van de ATG spiegels en de anti ATG titers voor hun rekening. Het engelengeduld van Henk van Lier van de Mathematisch Statistische Adviesafdeling tijdens analyse van de patientengegevens en zijn nuttige adviezen voor de uiteindelijke presentatie van de gegevens heb ik zeer op prijs gesteld. Ook Wim Lemmens en Albert Reintjes van deze afdeling hebben de nodige telefoontjes te verwerken gekregen. Wegwijs in de doolhof van computers en computertalen ben ik gemaakt door Jan van Rens, die tevens de eerste programma's voor het opslaan van patientengegevens maakte. De tekeningen van dit proefschrift werden met zorg vervaardigd door Cees Nicolasen van de afdeling medische illustratie en gefotografeerd door medewerkers van de afdeling medische fotografie. De heer E. de Graaff van de medische bibliotheek is mij steeds behulpzaam geweest bij het verzamelen van de literatuur. Tenslotte wil ik Ilse Hilgers-Biermans bedanken voor de enorme inzet en nauwgezetheid bij het typen en persklaar maken van het manuscript.

CURRICULUM VITAE

De schrijver van dit proefschrift werd geboren op 4 januari 1948 te Groningen. In 1966 werd het eindexamen HBS-B behaald aan de Rijks HBS te Leeuwarden. Aansluitend studeerde hij geneeskunde aan de Rijksuniversiteit te Groningen en behaalde het doctoraalexamen in 1971 en het artsexamen in 1973. Vervolgens vervulde hij als bataljonsarts zijn militaire dienstplicht. Vanaf 1 december 1974 volgde hij de opleiding tot internist, aanvankelijk in het St.Josephziekenhuis te Eindhoven (hoofd van de opleiding Dr. P.F.L. Deckers) en vanaf 1 april 1978 aan de universiteitskliniek voor Inwendige Ziekten van het Sint Radboudziekenhuis te Nijmegen (hoofd destijds Prof.dr. C.L.H. Majoor† en sinds januari 1980 Prof.dr. A. van 't Laar). Op 1 december 1979 werd hij als internist ingeschreven in het specialistenregister. Vanaf maart 1981 is hij verbonden aan de afdeling nierziekten van de universiteitskliniek voor Inwendige Ziekten van het Sint Radboudziekenhuis te Nijmegen (hoofd Prof.dr. R.A.P. Koene). Hij is getrouwd met Stieneke van 't Hof en zij hebben 2 kinderen: Arjen en Nienke.

STELLINGEN

1. Een acute rejectie van een niertransplantaat kan het beste behandeld worden met antilymfocytenglobuline.

2. Het gebruik van antilymfocytenglobuline ter voorkoming van acute rejecties van niertransplantaten is obsoleet.

3. De incidentie van acute rejecties is bij DRw6-positieve transplantaatontvangers verhoogd. Desalniettemin kan door behandeling van deze rejecties met antilymfocytenglobuline een slechtere transplantaatoverleving voorkomen worden

4. Acute transplantaatrejecties die niet of slecht reageren op hoge doses prednison, kunnen in het merendeel van de gevallen wel goed bestreden worden door een aanvullende behandeling met antilymfocytenglobuline.

5. Het metabole defect bij cystinurie kan hersteld worden door een niertransplantatie.
   Hoitsma AJ, Koene RAP, Trijbels FJM, Monnens LAH Disappearance of cystinuria after renal transplantation JAMA 1983, 250 615

6. Matige hydratie van de ontvanger in combinatie met toediening van mannitol maakt postoperatieve tubulusnecrose na niertransplantatie tot een zeldzame complicatie.

7. Het therapeutisch effect van monoclonale muize-antistoffen bij de mens wordt niet zozeer bepaald door het complement-activerend als wel door het Fc-receptor bindend vermogen van de betreffende subklasse.
   Tax WJM, Willems HW, Reekers P, Capel PJA, Koene RAP Polymorphism in mitogenic effect of IgG1 monoclonal antibodies against T3 antigen on human T cells Nature 1983, 304 445

8. De vrees dat bij nierdonoren op langere termijn nierfunctiestoornissen zullen ontstaan door een focale segmentale glomerulosclerose in de resterende nier, lijkt ongegrond.
   Hoitsma AJ, Paul LC, van Es LA, Koene RAP Long-term follow-up of living kidney donors, a two centre study Neth J Medicine 1984, in press

9 Bij dialysepatienten met gestoorde blaasfunctie kan een niertransplantaat zonder problemen worden aangesloten op een ileocutaneostomie

Arendsen HJ, Hoitsma AJ, Debruyne FMJ Niertransplantatie bij patienten met een urineweg-omleiding Ned T Geneesk 1984, in druk

10 Een sterke, maar in ernst wisselende proteinurie bij een normaal serum albumine kan wijzen op de aanwezigheid van lichaamsvreemd eiwit in de urine

Hoitsma AJ, Koene RAP, Raes BCM Pathomimie met afwijkingen in de urine Ned T Geneesk 1982, 126 2281-2283

11 Wil de volleybalsport zeker bij de mannen – weer een enigszins aardig kijkspel worden, dan zullen er lengteklassen ingesteld moeten worden

12. Het valt te betreuren dat de meeste Nederlanders met de Friese vlag alleen hun koffie op smaak brengen.

Nijmegen Andries Hoitsma